Identification of prognostic burns-related indicators and microRNA biosignatures in burns patients with inhalation injury

by

Tarryn Kay Prinsloo

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Supervisor: Prof Kareemah Najaar
Co-supervisor: Dr Wayne George Kleintjes

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DECLARATION

I, **Tarryn Kay Prinsloo**, declare that the contents of this thesis represent my own unaided work, and that the thesis has not previously been submitted for academic examination towards any qualification. Furthermore, it represents my own opinions and not necessarily those of the Cape Peninsula University of Technology.

Signed:  **Date:** 24 July 2025

ABSTRACT

Burn injuries remain a major global public health concern, disproportionately affecting low- and middle-income countries (LMICs). The high incidence and adverse outcomes stem from both socioeconomic disparities and the complexity of thermal injury, which is further influenced by co-factors such as inhalation injury. This condition, caused by toxic smoke inhalation during thermal events, is strongly associated with increased mortality and morbidity due to its variable clinical presentation and delayed onset. Despite being one of the most important mortality co-factors, there are no standard diagnostic criteria, and those that do exist have numerous shortcomings that particularly impact resource-poor clinical settings. Early identification of inhalation injury could improve burn management by leveraging readily available clinical markers from medical records. Additionally, most burn patients require intravenous fluid administration as part of standard protocol, presenting an opportunity for non-invasive blood sampling for biomarker analysis. Ideal biomarkers require that they be stable, disease-specific, and quantifiable, a criterion met by small, single-stranded RNA molecules with the ability to regulate gene expression called miRNAs. The primary and overall aim of this study was therefore to identify burn-related clinical markers extracted from a selected cohort of burn patient's medical records and miRNA biomarkers from the patients' whole blood samples using sequencing and bioinformatics tools. Following ethical approval and patient consent, the medical record data were extracted, and corresponding whole blood samples were collected from burn patients (n=59) admitted to the Western Cape Provincial Adult Tertiary Burns Centre (WCPATBC) at Tygerberg Hospital, South Africa, between 23 April 2016 and 15 August 2017. Recorded burns-related variables included sociodemographic factors (i.e. gender, age, referral level, etc.), burn severity (i.e. % total body surface area [TBSA] burns, inhalation injury, complications, etc.), and clinical factors (i.e. burns intensive care unit length of stay [BICU LOS], partial gas pressures, lactate, etc.). Inhalation injury was stratified based on total ventilation days, with mild inhalation injury defined as ≥4 days and severe inhalation injury as ≥5 days.

The first aim was to evaluate whether the recorded medical data aligned with reported findings in South Africa and if it was comparable to data reported from LMIC clinical settings. This analysis facilitated the identification of trends associated with increased mortality risk and inhalation injury in these vulnerable groups. Descriptive statistics were reported as mean (95% CI) and/or frequency (%) and analyzed using IBM SPSS Statistics version 28. Results showed that the majority of patients admitted were male, of working-age adults (21-39 years), referred from local areas within the district, who had sustained flame burns, primarily on weekdays and during colder months. The majority had serious to maximum abbreviated burn severity index (ABSI) scores, <40% TBSA burn and severe inhalation injury. While most arterial blood gas (ABG) measurements were found to be within normal ranges, elevated PO_2 and lactate levels were observed. The overall mortality rate was 25.4%, with higher rates found among females, younger and older age groups, and in patients referred from distant districts and

clinics or community health centres. Elevated mortality rates were also observed in patients with flame burns, larger TBSA, severe inhalation injury, complications, longer ICU LOS, pre-admission ventilation, and ABG values with elevated lactate; and reduced pH, PaO_2, PCO_2, and base excess levels. These findings strongly aligned with previously reported South African and LMIC-based data, with worse outcomes demonstrated compared to high income countries (HICs). The findings reported in this section contribute to the limited national literature and data on burn patients and highlights the continued burden of burns (and disease) in LMICs, potentially due to economic constraints and limited specialized burn care. Identifying these trends may help identify prevention strategies adapted from HICs to reduce burn incidence. Although some of the burn injury findings varied compared to the reported literature, their prognostic value remains crucial in assessing survivability.

The second aim was to *(i)* assess burn-related factors in relation to mortality to determine specifically whether inhalation injury reported in this patient cohort was a potential significant mortality co-factor, and *(ii)* whether the findings corroborated previous reported studies. Relationships between burn-related variables, mortality, and inhalation injury were analyzed using Fisher's Exact test (association), Pearson's point biserial (r_{pb})/Spearman's correlation (rho) coefficient (correlation strength/direction), and partial least squares regression (predictive contribution via variable importance in prediction [VIP] values). Correlation strength was categorized from none to very strong, with VIP scores >0.5 indicating significant contribution and >1 demonstrating greater predictive power. Statistical analyses were conducted in IBM SPSS Statistics version 28, with significance set at $p<0.05$ (two-tailed). An average of 2.7 patients (CI: 2.3–3.4) sustained mild inhalation injury, while 11.2 patients (CI: 9.5–12.9) had sustained severe inhalation injury, with the latter associated with 93.3% mortality, a strong positive correlation (rho = 0.441), and a significant predictive contribution (VIP = 0.819). Additional mortality-correlated factors included referral setting, TBSA, complications, and BICU LOS. The multifactorial nature of burn outcomes would predictably see mortality correlate with multiple parameters, of which inhalation injury had a particularly strong impact. In alignment with previous reported studies, these findings confirm inhalation injury as a critical mortality co-factor, supporting the need for further research.

The third aim was to evaluate the relationship between burn-related variables and inhalation injury using the same statistical methods employed with the second aim. Identifying significant relationships would indicate which variables could potentially serve as the most effective prognostic markers. Analysis displayed notable positive correlations observed for lactate (rho=0.331), %TBSA (rho=0.357), complications (rho=0.690), and BICU LOS (rho=0.908), with complications and BICU LOS showing the strongest correlations and the highest predictive contributions (VIP=1.229 and 1.372, respectively). Initial in-hospital prognosis could benefit or be improved by considering markers that would immediately present on admission or shortly thereafter. Complications may therefore be more suitable for early prognosis, while prolonged BICU LOS could provide insight into the degree of progression.

Lactate levels, measured shortly after injury, may offer valuable prognostic information, based on the positive correlation observed with inhalation injury. However, further studies are required to determine the specific lactate level changes that correlate with particular pathological sequelae.

The fourth aim was to identify potential miRNA biomarkers from whole blood samples of the burns patients, which had been collected in parallel with the patients' medical records. Total RNA was extracted, and its quantity and quality were assessed using a NanoDrop and a Bioanalyzer, respectively. Thirty exemplars representing mild and severe inhalation injury were selected for sequencing, with 22 passing quality control (mild: n=8, severe: n=14) and sequenced using the Illumina NextSeq 550 platform. Reads were aligned to the human genome (GRCh38) with Bowtie in sRNAbench, and miRNAs were quantified as counts per million using sRNAde. Differential abundance analysis was conducted with EdgeR in R v4.1.2 and validated by DESeq2. Fisher's Exact test compared differentially expressed (DE) miRNAs between groups, and significance was set at Padj.<0.05 with fold changes |log2 (FC)| >1.5. Results displayed ten overlapping DE miRNAs that met the significance threshold, comprising nine up-regulated in severe injury and one down-regulated in mild injury. MiR-30a-5p, miR-15a-5p, and miR-21-5p had the highest degree rankings, targeting 734, 717, and 612 genes, respectively. These findings present a potential panel of miRNAs that may present a cocktail of inhalation injury prognostic markers. The identified miRNAs have also been demonstrated as having key roles in mechanisms of inflammation and apoptosis, as well as in conditions closely related to inhalation injury, despite varying methodology.

The fifth aim was identify the mRNA target genes of the DE miRNAs and determine the associated pathways that were regulated. This could demonstrate biological significance of the identified miRNAs relative to the pathways involved. Protein-protein interaction networks, analyzed using STRING and Cytoscape, identified key hub genes of the up-regulated (i.e. *TP53*, *AKT1*, *MYC*, *CTNNB1*, *EGFR*, *PTEN*, *JUN*, *STAT3*, *EP300* and *TNF*) and down-regulated miRNA (i.e. *TP53*, *MDM2*, *BCL2L11*, *CDK6*, *BBC3*, *GADD45A*, *BAX*, *FAS* and *RHOA*). Gene ontology, KEGG, Reactome, and PANTHER analysis revealed that the hub genes were mainly enriched in inflammatory and apoptotic pathways. The findings highlight the role of miRNAs and their target genes in pathways impacting potential hallmarks of inhalation injury. While further validation with larger cohorts and additional techniques is required, these preliminary results provide a strong foundation for identifying miRNAs with prognostic potential in inhalation injury.

Overall, the findings presented in this thesis demonstrated several key firsts in the field of inhalation injury prognostication, particularly within the South African and broader LMIC context. For the first time, prognostic burn-related indicators (TBSA, complications, BICU LOS, and lactate) were retrospectively identified as specific predictors of inhalation injury, rather than general burn mortality.

This research also represents the earliest application of human whole blood from burn patients to investigate miRNA expression profiles, proposing a novel panel of 10 differentially expressed miRNAs relating not only to the presence of inhalation injury but also to its severity (mild versus severe). While further validation is warranted, the strength of these preliminary findings was supported by consistent overlaps across statistical outcomes for burns-related medical-file-based indicators, while reinforced miRNA expression patterns were confirmed through dual bioinformatics pipelines, threshold filtering, and four independent enrichment platforms to ensure robust biological interpretation. Moreover, the use of whole blood, a readily accessible and clinically relevant biospecimen, may better represent systemic responses to injury, thereby improving the translational potential of the results. Collectively, this research presents the first integrated evaluation of retrospective clinical data alongside prospective molecular profiling within a single burn patient cohort for the purpose of inhalation injury prognostication. By combining these two dimensions of analysis, the study lays critical groundwork for incorporating both clinical and circulating biological markers—many of which have not been previously explored in this context—into existing diagnostic or prognostic frameworks. This approach not only broadens the spectrum of potential indicators but also sets the stage for the future development of comprehensive, multi-modal tools capable of enhancing the timely and precise assessment of inhalation injury.

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DEDICATION

To my mother and partner who have sacrificed and witnessed the culmination of this endeavour; and to those whose memory continues to inspire and guide me, even though they are no longer with us.

It is with deepest gratitude and respect that I thank you all for walking this path with me and for being a part of my story – in chapters past and pages yet to be written.

“If I have seen further, it is by standing on the shoulders of giants” – Isaac Newton.

ABBREVIATIONS AND ACRONYMS

ABG	Arterial blood gas
ABSI	Abbreviated burn severity index
AD	Alzheimer's disease
Ago2	Argonaute 2
AIS	Abbreviated injury score
AKI	Acute kidney injury
ALI	Acute lung injury
AKT1	AKT serine/threonine kinase 1
ANOVA	Appropriate analysis of variance
ARDS	Acute respiratory distress syndrome
BALF	Bronchoalveolar lavage fluid
BAX	BCL-2 associated X protein
BBC3	BCL-2 binding component 3
BCL-2	B--cell lymphoma-2
BCL2L11	B-cell lymphoma-2-like protein 11
BD	Base deficit
BE	Base excess
BICU	Burns intensive care unit
BOBI	Belgium outcome burn injury
BP	Biological process
CC	Cellular component
CCI	Charlson co-morbidity index
CCKR	Cholecystokinin receptor
CDK6	Cyclin-dependent kinase 6
CI	Confidence interval
CPT	Cape Town
CPUT	Cape Peninsula University of Technology
CO	Carbon monoxide
COHb	Carboxyhemoglobin
COPD	Chronic obstructive pulmonary disease
CSF	Colony-stimulating factor
CT	Computed tomography
CTNNB1	Catenin beta 1
DE	Differentially expressed
DGCR8	DiGeorge Syndrome Critical Region 8 Protein
DV	Dependent variable
ECs	Endothelial cells

EGFR	Epidermal growth factor receptor
EP300	E1A-binding protein p300
ER	Estrogen receptor
ERK	Extracellular signal-regulated genes
ETI	Endotracheal intubation
FAS	Fas cell surface death receptor
FC	Fold change
FDR	False discovery rate
FLAMES	Fatality by longevity, APACHE II, measured extent of burns and sex
FOB	Fiberoptic bronchoscopy
FOL	Fiberoptic laryngoscopy
GADD45A	Growth arrest and DNA damage-inducible protein GADD45 alpha
GO	Gene ontology
HCN	Hydrogen cyanide
HS	Hypertrophic scarring
HWREC	Health and Wellness Research Ethic Committee
ICU	Intensive care unit
IL	Interleukin
IQR	Interquartile range
IV	Independent variable
JAK/STAT	Janus kinase/signal transducers and activators of transcription
JUN	Jun proto-oncogene, AP-1 transcription factor subunit
KEGG	Kyoto Encyclopedia of Genes and Genomes
LA50	Lethal Area fifty
LMIC	Low- and middle-income countries
LOS	Length of stay
LPS	Lipopolysaccharides
MAPK	Mitogen-activated protein kinase
MCODE	Molecular complex detection
MDM2	Murine double minute-2
MF	Molecular function
mRNA	Messenger RNA
miRNA or miR	MicroRNA
MYC	Myc myelocytomatosis oncogene
NF-κB	Nuclear factor kappa
NGS	Next generation sequencing
ncRNA	Non-coding RNA
nt	Nucleotides
NO	Nitric oxide

NOS	Nitric oxide synthase
NOTCH	Neurogenic locus notch homolog protein
O_2^-	Superoxide anion
OD	Optical density
OH^-	Hydroxyl ions
$ONOO^-$	Peroxynitrate
PaO_2	Partial pressure of arterial oxygen
PaO_2/FiO_2 or P/F	Arterial oxygen tension to inspiratory oxygen fraction
PARP	Poly-(ADP ribose) polymerase-1
PBI	Prognostic burn index
PCO_2	Partial pressure of arterial carbon dioxide
PI3K	Phosphoinositide 3-kinase
PPI	Protein-protein interaction
Pre-miRNA	Precursor miRNA
Pri-miRNA	Primary miRNA
PLS	Partial least squares
PMN	Polymorphonuclear
PTEN	Phosphatase and tensin homolog
QC	Quality check
RADS	Radiologist's score
Ras	Rat sarcoma
RCCH	Red Cross Children's Hospital
rho	Spearman's Rank correlation co-efficient
RHOA	Ras homolog family, member A
RIN	RNA integrity numbers
RISC	RNA-induced silencing complex
RNAi	RNA-mediated interference
RNA seq	RNA sequencing
RNS	Reactive nitrogen species
ROCK	Rho-associated protein kinase
ROS	Reactive oxygen species
r_{pb}	Pearson's point-biserial correlation co-efficient
RT-qPCR	Reverse transcription polymerase chain reaction
QC	Quality control
SA	South Africa
SaO_2	Oxyhemoglobin saturation
SI-ALI	Smoke inhalation-induced acute lung injury
SMC	Smooth muscle cells
snc	Small non-coding

SOLiD	Sequencing by oligonucleotide ligation and detection
SpO_2/FiO_2 or S/F	Arterial saturation or standard pulse oximetry saturation
STAT3	Signal transducer and activation of transcription 3
STRING	Search tool for the retrieval of interacting genes/proteins
TBH	Tygerberg Hospital
TBSA	Total body surface area
TGF	Tissue growth factor
TNF	Tissue necrosis factor
TP53	Tumor protein 53
UTR	Untranslated region
VE	Vascular endothelial
VIP	Variable of importance in the projection
WCPATBC	Western Cape Provincial Adult Tertiary Burns Centre
WHO	World Health Organization

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CHAPTER ONE

INTRODUCTION

1. Inhalation injury

1.1. Global burden of disease and epidemiology

The World Health Organization (WHO) estimated that 180 000 deaths per year were attributed to burns, describing this observation as a global public health problem (World Health Organization, 2018a) with a loss of 10 million disability-adjusted life years by 2016 (World Health Organization, 2018b). A large fraction of the reported mortalities occurred in low- and middle-income countries (LMICs) where WHO African and South-East Asia regions accounted for approximately two-thirds (World Health Organization, 2018a). There was a clear, disproportionate, increased burden and prevalence of this injury type in LMICs, which was further supported by a near ten-fold incident increase compared with high-income countries (1.3/100 000 versus 0.14/100 000) (Gupta et al., 2014; Institute for Health Metrics and Evaluation, 2012; World Health Organization, 2008). Smolle and associates, (2017) assessed trends in burn epidemiology that indicated a consistent downward trend of burn incidence and severity, hospital length of stay (LOS), and mortality rates in developed countries. These were reportedly attributed to legislative changes, successful prevention programs, increased workplace safety (Duke et al., 2012), advances in burn treatment (Harats et al., 2016) and infection control (Al-Shaqsi et al., 2013). Furthermore, referral of burn injuries to specialized facilities greatly contributed to the observed trend (Smolle et al., 2017; Pegg, 2005). LMICs were commonly devoid of the latter advances, and therefore, the impact of burns management and treatment was likely to be more pronounced in these settings with the limited resource availability (Agbenorku et al., 2019). Allorto and co-workers, (2018) focused on quantifying the extent of resource deficits from seven out of the nine provinces in South Africa (SA). Their findings suggested deficits in the infrastructure and staffing available to treat burn injuries along with a need for outreach programs, protocols, and improving theatre efficiency (Allorto et al., 2018). Despite the country's economic growth, burn care services in SA differs according to infrastructure and staff organization, and clinical management (Rode et al., 2014). The reported deficiencies potentially played a role as contributing factors (Allorto et al., 2018) in the reported mortalities from burn units in SA (Cloake et al., 2017; Jugmohan et al., 2016; Den Hollander et al., 2014).

SA had been subjected to a constant influx of migrant workers spanning both rural and urban areas. This resulted in increased impoverished sectors governed by poor housing conditions (informal settlements), overcrowding, and greater dependence on unsafe energy sources (den Hollander et al., 2014; Kimemia et al., 2014). The informal dwellings ("shacks"), in addition, were comprised of highly flammable materials and built in close proximity to each other, thus creating environments for expeditious spread and catastrophic conflagrations (Kimemia et al., 2014; Peck et al., 2008). The incidence of "shack" fires was associated with 16.7% of burn admissions (Cloake et al., 2017), demonstrating a potential link to

burn hospitalization and mortality associated with informal settlements in SA (Singh et al., 1997; Forjuoh et al., 1995). In Cape Town (CPT), SA, these unfavourable conditions were closely linked to specific, vulnerable population groups (Parbhoo et al., 2010; Van Niekerk et al., 2009) and continued to be associated with lower socio-economic status and poorer overall health status (Day & Gray, 2003) that perpetuated conditions for more devastating burn outcomes (Edelman et al., 2007). Although clinical and epidemiological data in SA were limited, it was estimated that 3.2% of the population reported thermal injury annually (Allorto et al., 2016; Rode et al., 2014) and the associated mortality rate ranged between 5-16% (Cloake et al., 2017; Jugmohan et al., 2016; den Hollander et al., 2014).

1.2. Smoke inhalation injury as a co-factor of mortality

Elevated mortality and morbidity rates were not only attributed to the thermal injury itself, but also to the presence of additional cofactors (Douglas et al., 2015; You et al., 2014; Antonio et al., 2013). Advanced age (>60 years) and total body surface area (TBSA <40%) were reported to directly and adversely impact burn outcomes (Antonio et al., 2013; El-Helbawy & Ghareeb, 2011; Brusselaers et al., 2010); however, inhalation injury was highlighted as the most important cofactor (American Burn Association, 2019; You et al., 2014; Lipovy et al., 2011). Inhalation injury has been defined as damage to the respiratory passages after exposure to the toxic smoke inhalants during a thermal event (e.g. domestic fire, explosion, self-immolation, steam accidents, etc.) (Gill & Martin, 2015; Woodson, 2009). In the incidence of burns, smoke had a greater ability to disperse heat than dry air, which impaired the upper airways from efficiently dissipating the heat (Antonio et al., 2013). This damage physically manifested as upper airway obstruction (Sheridan, 2016) that triggered progressive inflammation in the airways (Antonio et al., 2013). The clinical outcomes of this inhalation injury degree were less severe compared to when the lower airways and pulmonary parenchyma were affected. The causative agents for severe injury were the smoke inhalants that consisted of partially combusted, aerosolized chemicals and particles often released from common household and domestic items (Shubert & Sharma, 2018; Sheridan, 2016). Carbon monoxide (CO) and hydrogen cyanide (HCN), were the most destructive gases released that not only resulted in non-specific clinical outcomes and misdiagnosis (Foncerrada et al., 2018), but also had a greater effect on morbidity and mortality. Varying smoke constituents was one of several reported challenges in predicting and diagnosing this type of injury; which also included injury location that may vary according to the origin of the fire, the duration of exposure, and underlying respiratory conditions (Walker et al., 2015; Traber et al., 2007).

The prevalence of inhalation injury in burn patients varied from a low of 6% prevalence (Latenser et al., 2007) to a reported 46% (El-Helbawy & Ghareeb, 2011). Studies that assessed burn outcomes at the Tygerberg Hospital (TBH)'s Western Cape Provincial Adult Tertiary Burns Centre (WCPATBC) in CPT reported a prevalence of 26.7%, 31.2% and 47% that were predominantly attributed to flame origin and/or shack fire events (Boissin et al., 2019; Cloake et al., 2017; Maritz et al., 2012). Inhalation injury was therefore present in least a third of the time in burn patients from resource-poor environments.

Regardless of incidence disparity, there was a clear trend in the relationship between inhalation injury and mortality. As an isolated condition, it was already reported to increase mortality by 20%; however, concomitant occurrence with cutaneous burns resulted in even higher rates (You et al., 2014; Dries & Endorf, 2013; Shirani et al., 1987). Studies consistently reported lower fatalities in patients with burns only compared with those that had concomitant inhalation injury. The fold increases observed were always above 2.0 and even reached a 5.8-fold (Karimi et al., 2016; El-Helbawy & Ghareeb, 2011; Suzuki et al., 2005; Muller et al., 2001; Shirani et al., 1987). These South African-based studies indicated that inhalation injuries greatly impacted the chances of survival as a single factor as well as with accompanying co-morbidities.

1.2.1. Complications associated with inhalation injury

The presence of inhalation injury has been associated with an elevated risk for complications (Shubert & Sharma, 2018) that greatly affect injury outcome (Rue III et al., 1995). Despite medical advances, resultant subsequent morbidity and mortality rates remained high (Albright et al., 2012; Lipovy et al., 2011). These complications could be acute or delayed and varied according to injury management, degree, and chronic inflammation (Cancio, 2009). Both cutaneous burns and inhalation injury resulted in a cascade of pathophysiological events, in which the combined and resultant immunocompromising effects predisposed patients to both wound and respiratory complications (Liodaki et al., 2015; Edelman et al., 2007). Those more frequently observed included pulmonary infections, sepsis, smoke inhalation-induced acute lung injury (SI-ALI), and acute respiratory distress syndrome (ARDS). Pulmonary infections, particularly pneumonia, were the most common reported complication in critical care (Liodaki et al., 2015; de La Cal et al., 2001). Inhalation injury not only increased the predisposition to pneumonia, but also resulted in a 21-fold increased risk (Rue III et al., 1995; Haley et al., 1981) was linked to the standard injury management, i.e. intubation or mechanical ventilation support (Cobley et al., 1999; Thompson et al., 1986). Early diagnosis was therefore imperative to adjust treatment and also to potentially mitigate for the associated mortality risk of 60% (Dries & Endorf, 2013; Edelman et al., 2007). Pneumonia was commonly reported in burn patients who developed sepsis (Rech et al., 2019). With the prevalence of sepsis in these burn patients ranging between 3-30% (Jeschke et al., 2015) which attributed to 65% of deaths in adults (Sharma et al., 2006). A direct outcome of sepsis was multiple organ dysfunction syndrome, which was the leading cause of burn fatalities in patients who had initially survived the thermal insult (Greenhalgh, 2017).

The observed multiple organ failure was also frequently linked to SI-ALI (Guo et al., 2019; Veeravagu et al., 2015). ALI was part of the spectrum of inhalation injury-associated respiratory conditions and was reported to be a less severe form of ARDS (Kimmel & Still, 1999). The severity distinction between ALI and ARDS was illustrated by the difference in oxygenation or the extent of hypoxia with ARDS resulting in the more severe outcome (Kimmel & Still, 1999). However, ARDS was the leading complication associated with severely burnt patients (Lam & Hung, 2019; Dancey et al., 1999), which

also resulted in multisystem organ failure and death (Matthay et al., 2012). The progression of ARDS was triggered by pneumonia (direct) and sepsis (indirect), respectively (Laycock & Rajah, 2010; Ware & Matthay, 2000) and both factors were causative factors for ALI (Mackay & Al-Haddad, 2009). A reported independent risk factor for ARDS was the presence of inhalation injury (Liffner et al., 2005) and when compared to patients with only cutaneous injuries, the onset was earlier, severity greater (Rawal et al., 2018) and the mortality rates higher (Lam & Hung, 2019; Silva et al., 2016). It was postulated that the presence of inhalation injury should at the very least be considered when ARDS/ALI was present, since it was a proposed part of the direct sequelae (Oh et al., 2012).

1.3. Pathophysiology of inhalation injury

The mechanisms by which inhalation injury exerts its effects played a crucial role in the location and development of the damage that manifested. Based on localization, inhalation injury could affect the upper and lower airways, as well as, the lung parenchyma (Woodson, 2009; Traber et al., 2007). Damage to the oropharyngeal mucosal structures predominantly resulted from heat injury, while secondary damage was reportedly caused by water-soluble chemicals (Rong et al., 2011; Cox et al., 2009). During the initial 12 hours post-inhalation injury, the resultant microvascular changes (Mlcak et al., 2007) caused acute inflammation (Dries & Endorf, 2013; Prien & Traber, 1988) with edema presence already reported after a few hours (Shubert & Sharma, 2018; Dries & Endorf, 2013). Tissue edema developed and progressed over the initial 24-36 hours after insult (Gill & Martin, 2015). These conditions were promoted by thermal injury through the initial release of neuropeptides and activation of the complement cascade (Traber et al., 2012; Cox et al., 2009; Friedl et al., 1989). This, in turn, triggered the release and/formation of inflammatory mediators, histamine, and subsequent pathological by-product agents that not only promoted acute inflammation and edema, but also amplified it (Traber et al., 2012; Cox et al., 2009). Along with the formation of secretions such as mucus and slough, edema impeded airflow, thereby impairing oxygenation status (Shubert & Sharma, 2018; Dries & Endorf, 2013). Without intervention, injury progression included obstruction of the distal airways, atelectasis, and impaired gaseous exchange (Rehberg et al., 2009; Cox et al., 2003). Upper airway injury could therefore be life-threatening soon after insult, but was also dependent on the severity and distribution of cutaneous injury, solubility of the chemical inhalants, and the duration of exposure to smoke (Jones et al., 2017; Haponik et al., 1987). Prolonged exposure to smoke and the chemical inhalants present was associated with more severe injuries that presented in the lower airway and alveoli. Injury to the lung parenchyma may even occur in the absence of lower airway damage, an outcome reported in patients exposed to chemical toxins with low water solubility (Shubert & Sharma, 2018). The smoke-related toxins stimulated the vasomotor and sensory nerve endings (vagus nerve C-and Aδ fibers) in the tracheobronchial area, causing neuropeptide release (Fontán et al., 2000). The neuropeptides reportedly involved in inhalation injury-induced inflammatory response are thought to do so through their tachykinin-related function (Walker et al., 2015; Tai & Baraniuk, 2002). Substance P (Fontán et al., 2000) and calcitonin gene-related peptide specifically (Lange et al., 2009), were the reported neurotransmitters that resulted in both

the initial and enhanced inflammatory responses (Fontán et al., 2000).

The role of these neuropeptides in burn-induced acute lung injury and the neurogenic inflammation was also strongly linked to the induced release of pro- and anti-inflammatory mediators and the modulation of immune cells (Sio et al., 2008; Brain et al., 1985; Morris et al., 1984). Aggregation of the immunoinflammatory cells stimulated the release of proinflammatory mediators, such as chemokines and cytokines (Albright et al., 2012; Levite, 1998; Torii et al., 1997); and triggered additional pathways that progressed to pulmonary damage (Sio et al., 2008). Dysregulation in cytokine and chemokine levels induced by inhalation injury was also hypothesized to cause immune dysfunction and has also been linked to burns-related mortality cases (Davis et al., 2012). Cytokine and chemokine proteins increased cellular migration into the respiratory microvasculature by affecting vascular endothelial cells (ECs) and subsequently elevated permeability and vasodilation that modulated fluid leakage (Ravage et al., 1998). Under these conditions, ECs release adhesion molecules (Pober & Cotran, 1990; Osborn et al., 1989) for polymorphonuclear (PMN) leukocyte recruitment and activation (Ravage et al., 1998), or direct altered cell structure (Maruo et al., 1992). PMN accumulation resulted in the complement cascade activation and further EC stimulation, while its increased infiltration has also been linked to additional neuropeptide release (Sio et al., 2008; Puneet et al., 2006). Activated PMNs were reported to translocate from the pulmonary circulation to the microvasculature due to the elevated permeability and the inability to traverse the capillaries (Traber et al., 2007). The altered microvasculature at numerous pulmonary areas resulted in increased blood flow that allowed the activated PMNs and cytokines to be rapidly transported to the lung (Walker et al., 2015). This increased filtrate reportedly played a pivotal role in lung inflammatory responses and initiated a cascade of adverse events (Traber et al., 2007). Along with vasodilation and increased blood flow, the inflammatory response was not only exacerbated by the elevated infiltrate (Walker et al., 2015), but was also suggested to contribute to pulmonary edema formation (Abdi et al., 1991; Kramer et al., 1989), cast and exudate development (Walker et al., 2015; Murakami & Traber, 2003), and subsequent hypoxemia as a consequence of ventilation-perfusion mismatch (Murakami & Traber, 2003).

The activated inflammatory cells also adhered to EC's adhesion molecules (Rehberg et al., 2009; Sakurai et al., 1999) and blocked the accessibility and expression of heme groups. Heme groups are responsible for scavenging and metabolizing nitric oxide (NO); however, the impairment in heme expression leads to excessive production of NO (Murakami & Traber, 2003), reported as a product of numerous cells, including ECs and macrophages (Murakami & Traber, 2003). NO production catalysed by the enzyme group nitric oxide synthases (NOS) are also found in lung epithelial cells (Watkins et al., 1997) and in neutrophils (Greenberg et al., 1998). Three isoforms, namely NOS–1 (neuronal/nNOS), NOS–2 (inducible/iNOS), and NOS–3 (endothelial/eNOS) have been located in various cells (Murakami & Traber, 2003; Steudal et al., 2000; Szabó et al., 1995). In excess, NO would act as a free radical and react aggressively with oxygen free radicals produced by activated neutrophils (Traber et al., 2012;

Murakami & Traber, 2003). The resultant reactive oxygen species (ROS) produced: Superoxide anion (O_2^-), hydrogen peroxide and hydroxyl ions (OH^-); resulted in oxidative stress (Weyker et al., 2013; Park et al., 2004), increased neutrophil chemotaxis and elevated cytokine production (Walker et al., 2015; Lange et al., 2012). In addition, NO reacted with O_2^- to form the reactive nitrogen species (RNS), peroxynitrate ($ONOO^-$) and OH^-, (Freeman, 1994), which was reported to cause DNA damage (Traber et al., 2012). $ONOO^-$ was also reported to activate poly-(ADP ribose) polymerase-1 (PARP), which is important for DNA repair. DNA repair function of PARP require a large amount of chemical energy and inhalation injury reportedly overstimulates its synthesis resulting in ATP depletion, cell damage (Gill & Martin, 2015; Lange et al., 2010; Espinoza et al., 2007) and necrotic cell death (Liaudet et al., 2002). In addition, PARP also stimulated nuclear factor kappa B (NF-κB) (Jagtap & Szabó, 2005; Chiarugi & Moskowitz, 2003) which was reported to upregulate inflammatory mediators (Li et al., 2003). Subsequently, a positive feedback mechanism also caused upregulation of inflammatory mediators, which could result in PMN activation and attraction; NO, ROS, and RNS formation (Yamaza et al., 2003); as well as DNA damage followed by PARP and NF-κB reactivation (Rehberg et al., 2009).

The time-related development and progression of the events reported, led to a delay in the damage to the lung parenchyma (alveoli) in burn patients (Rehberg et al., 2009). This neurogenic inflammatory response continuously stimulated the activation and chemotaxis of leukocytes, which ultimately resulted in alveoli injury by amplifying the previously described pathological sequelae (Rehberg et al., 2009). Derangements for this degree of injury were owed mainly to the bronchial hyperemia with subsequent augmented transvascular fluid flux (lymph fluid) that in turn promoted edema and airway exudation (Jones et al., 2017; Mlcak et al., 2007). The summative effect of these conditions also impaired the functions of surfactant (Sousse et al., 2015; Mlcak et al., 2007), alveolar macrophages and mucociliary clearance (Al Ashry et al., 2016; Mlcak et al., 2007); in addition to the observed reduced lung compliance (Jones et al., 2017; Mlcak et al., 2007) and increased airway resistance (Sousse et al., 2015). Parenchymal damage was therefore characterized by alveolar damage and atelectasis that presented with diffuse edema, epithelial cell death and sloughing (Shubert & Sharma, 2018). The basis of pulmonary dysfunction was the ability of all the factors to not only result in the observed outcomes, but also exacerbate it. The adverse outcomes were therefore not dependent on a single event but resulted from concomitant activation of numerous modulators that amplified the inflammatory process, consistently co-stimulated the production of pathological-inducing agents and aggravated edema formation.

1.4. Diagnostic criteria and modalities of inhalation injury

1.4.1. Medical history and clinical evaluation

The diagnosis and treatment of inhalation injury have varied among clinicians, but have traditionally been made based on a combination of subjective and objective measures, with a high index of suspicion (Kim et al., 2017; Jones et al., 2017; Enkhbaatar et al., 2016). This provided pertinent information aiding

in potentially identifying the host response and co-morbidities associated with inhalation injury for suspected cases (Jones et al., 2017). Without the existence of a uniform criterion, consensus would initially rely on the latter measures followed only then by the use of diagnostic modalities for confirmation (Woodson, 2009). History-related parameters that would be considered during evaluation included the mechanism and duration of exposure, quality of inhaled irritants, location of thermal event, loss of consciousness and disability (Enkhbaatar et al., 2016; Walker et al., 2015; Toussaint & Singer, 2014). The clinical factors included facial burns, singed nasal or facial hair, presence of carbonaceous material in the sputum or on the face, soot in the oral cavity or proximal airways, vocal alterations, and indications of airway obstruction (Enkhbaatar et al., 2016; Sheridan, 2016; Walker et al., 2015). Although many of the factors may overlap in a single incident, some common signs and/or symptoms may not be present. Therefore the presence of inhalation injury to a certain degree could be subjective, however, its confirmed presence or absence could not be guaranteed (Cancio, 2009). Moreover, the associated latency (Masanès et al., 1995) would not only inaccurately reflect the presence and extent of the injury, but could also cause inconsistencies in identifying abnormal findings using various diagnostic tools (Chou et al., 2004; Lee-Chiong Jr, 1999).

1.4.2. Mechanical diagnostic modalities

Fiberoptic bronchoscopy (FOB) was established as the current 'gold standard' diagnostic modality for inhalation burn injury (Walker et al., 2015; Yang et al., 2011; Hunt et al., 1975) to determine the presence of related sequelae, e.g. hyperaemia, edema, soot, etc. (Jones et al., 2017; Woodson, 2009) and the development of early pneumonia (Carr & Crowley, 2013). The high diagnostic precision rate (approximately 100%) of FOB (Souza et al., 2004; Bingham et al., 1987) had the added benefit of detecting anatomical alterations before changes in gaseous exchange occurred (Souza et al., 2004; Lee-Chiong Jr, 1999), but was reportedly unable to visually detect beyond obstructive lesions (Naidich et al., 1997) and efficiently evaluate the lower airway and potential mucosal damage to certain areas (Jones et al., 2017; Walker et al., 2015; Hunt et al., 1975). Furthermore, edema or erythema may not appear on the initial bronchoscopic results (Souza et al., 2004; Haponik et al., 1987). When patients were unsuitable for FOB or in its absence, other diagnostic tools have been utilized which included virtual bronchoscopy using spiral computed tomographic scanners, fiberoptic laryngoscopy (FOL), chest radiographs (x-rays), computed tomography scans, Xenon 133 ventilation-perfusion scans, and pulmonary function tests. While these modalities identified facets of inhalation injury, their limitations were related to the inability to determine lower airway and parenchymal damage which typically presented in severely injured patients (Jones et al., 2017; Muehlberger et al., 1998; Talmi et al., 1996). Even in the presence of marked lung damage (Woodson, 2009; Wittram & Kenny, 1994), these challenges resulted in clinical observations often appearing normal after admission (Gill & Martin, 2015; Peters, 1981).

Many of the modalities were not included as routine protocol due to the requirement of an active patient participation (Ranu et al., 2011; Woodson, 2009; Teixidor et al., 1983) in which procedures were not well tolerated or considered invasive (Muehlberger et al., 1998; Naidich et al., 1997; Schall et al., 1978). Moreover, the need for patients to be physically transported for evaluation potentially delays the much-needed treatment (Woodson, 2009; Young & Moss, 1989). An additional deterrent was the possibility of false positives or negatives due to decreased sensitivity and specificity in the presence of confounding pre-existing and late progression of lung diseases (Woodson, 2009; Mlcak et al., 2007). It was suggested that greater diagnostic potential would be achieved when the use of modalities was combined (Woodson, 2009; Cioffi Jr et al., 1991; Schall et al., 1978) or, individual implementation would be more useful in assessing the progress of inhalation injury and its complications, as opposed to being utilized as a tool for initial diagnosis (Souza et al., 2004; Casper et al., 2002; Lund et al., 1985). Perhaps the most applicable limitation, relevant to current clinical settings, related to the commonly observed logistic and fiscal costs (Enkhbaatar et al., 2016; Tanizaki, 2015; Shiau et al., 2003; Hunt et al., 1975). Burn Centres may have reported limited resources and a lack of readily available equipment such as bronchoscopes, along with the trained personnel required to operate the equipment (Palmieri & Gamelli, 2012; Muehlberger et al., 1998).

1.4.3. Blood gas analysis

The diagnosis of inhalation injury, as determined by arterial blood gas (ABG) analysis, involved blood sampling and the subsequent measurements of the partial pressures of arterial oxygen (PaO_2) and arterial carbon dioxide (PCO_2), as well as oxyhemoglobin saturation (SaO_2) and carboxyhemoglobin (COHb) levels (Crapo, 1981). Impaired gaseous exchange (Demling, 2008), injury to the upper or lower airway, and lung parenchymal damage (Traber et al., 2007; Cohen & Guzzardi, 1983) were consequences reported when these parameters were altered. Altered ABGs were also indicative of hypoxemia, specifically characterized by reduced PaO_2 or SaO_2 reported in patients with inhalation injury (Tripathi et al., 1983; Crapo, 1981). ABGs, in addition, also provided acid-base status post-injury indicative of cellular metabolic alterations (Beresneva et al., 2014; Crapo, 1981) by monitoring pH, base excess or deficit (BD), and lactate concentration (Mackutwa et al., 2021). Elevated lactate and BD, which in turn lowered pH, were more commonly associated in patients with severe burns and poorer outcomes (Mackutwa et al., 2021). Literature showed that the majority of studies investigated these parameters as potential markers for mortality, but research associated with inhalation injury as the primary outcome were scarce. Studies have linked altered lactate and/or base-related buffers to conditions associated with inhalation injury such as sepsis, shock (Mokline et al., 2017; Kraut & Madias, 2014), impeded perfusion (De Lucas et al., 2020), tissue hypoxemia, impaired oxygenation (Kraut & Madias, 2014; Kraut & Kurtz, 2006), and exposure to cyanide and CO (Kennedy & Cahill, 2020; Kraut & Madias, 2014). This could provide the basis for using these parameters as clinical markers for the potential presence and degree of inhalation injury. Additionally, the arterial oxygen tension to inspiratory oxygen fraction (PaO_2/FiO_2 or P/F) ratio has been suggested as a more reliable marker for injury severity (Erickson et al., 2009). A

reduced P/F measurement corresponded with inhalation injury-related outcomes based on its ability to quantify gaseous exchange anomalies and stratify hypoxemia (Rice et al., 2007; Piccinni et al., 2006). To mitigate the invasively acquired P/F values, arterial saturation or standard pulse oximetry saturation (SpO_2)/FiO_2 (S/F) ratio was reportedly utilized (Merlani et al., 2001; Pilon et al., 1997; Roberts et al., 1991). S/F ratios determined the extent of hypoxia (Bilan et al., 2015), illustrated a strong correlation with altered PaO_2 (Perkins et al., 2003; Yamaya et al., 2002) and notably corresponded to the simultaneously obtained P/F ratio in the presence of ALI and ARDS (Rice et al., 2007). The S/F ratio did, however, not indicate related $PaCO_2$ levels or acid-base status (Kwack et al., 2018; Rice et al., 2007) that typically accompany inhalation injury (Mlcak et al., 2007; Park et al., 2006).

Oxygen-related trends were also determined using standard pulse oximetry and CO-oximetry which avoided blood sampling with traditional ABG analysis. Oximetry evaluation uses differential spectrophotometric absorbance measurements (Chan et al., 2013; Sinex, 1999) that determine the amount of Hb-oxygen saturation levels (Chan et al., 2013; Mack, 2007), the development of hypoxia that follows injury (Pretto et al., 2014; Jubran & Tobin, 2013), and COHb levels (blood CO saturation) associated with toxin (CO/HCN) exposure (Lindner & Exadaktylos, 2013; Palmieri & Gamelli, 2012; Demling, 2008). The latter parameter provided information related to gas exchange, metabolic status, hypoxemia, and COHb levels (Dries & Endorf, 2013; Hampson, 2008; Mack, 2007). Comparatively, CO would bind more readily to haemoglobin than oxygen forming COHb; therefore, the reduced oxygen capacity of blood and resultant oxygen delivery to the surrounding tissues (Dries & Endorf, 2013; Kealey, 2009) could cause tissue hypoxia, acidosis and direct cellular damage (McCall & Cahill, 2005; Moore et al., 1991). The absence of altered COHb, however, did not exclude the likelihood of toxin exposure or injury occurrence in general (Symington, 1978) and even in the presence of small cyanide concentrations (5-10%) in blood were considered abnormal and resulted in significant damage (Trunkey, 1978). Thus, the possibility of inhalation injury being present was still high (Thom et al., 2010; Weaver, 2009; McCall & Cahill, 2005). ABG analysis was reportedly highly beneficial in diagnosing inhalation injury; however, the disadvantages would make it difficult to have major prognostic value due to delayed sequalae. Regardless of extensive symptomology, the potential of ABG outputs within normal ranges was still reported (Ernst & Zibrak, 1998; Young & Moss, 1989), and the presence of inhalation injury could not entirely be excluded (Wittram & Kenny, 1994; Cohen & Guzzardi, 1983). Difficulty in determining the true extent of inhalant toxicity has also been observed with varying time measurements, along with delayed testing versus incidence occurrence (Dries & Endorf, 2013; McCall & Cahill, 2005; Crapo, 1981). It was proposed that perhaps the merits of ABG as a diagnostic modality were overshadowed by its unavailability in clinical settings (Piatkowski et al., 2009).

1.4.4. Prognostic scoring systems

1.4.4.1. Grading systems for inhalation injury

Numerous grading systems have been used to predict injury outcomes based on co-factors associated with burns; however, these have primarily utilized inhalation injury as a prognostic parameter, rather than predicting the injury itself. Strong prognostic value in predicting burns-related mortality was observed; however, no standard system has officially been assigned for severity grading, prognosis, or diagnosis of inhalation injury (Walker et al., 2015; Woodson, 2009). The current scoring systems were derived from diagnostic outputs that related severity to adverse outcomes, which were dependent on the range of scores that it fell within. Based on FOB measurements, the abbreviated injury score (AIS) evaluated injury severity (Endorf & Gamelli, 2007) that reportedly corresponded with impaired gas exchange and mortality (Albright et al., 2012; Mosier et al., 2012; Hassan et al., 2010). AIS values ranged between 0 and 4, where 0 was assigned in the absence of inhalation injury and the maximum score of 4 indicated severe injury. Grades 1, 2, and 3, respectively, represented the presence of mild, moderate, and severe injury (Albright et al., 2012; Endorf & Gamelli, 2007). Increasing values correlated with progressively worse clinical findings (Endorf & Gamelli, 2007), while grades 2-4 have also been associated with reduced survival rates compared to the lower scores. Confounding reports have noted variable results in its predictive potential (Albright et al., 2012; Mosier et al., 2012; Woodson, 2009) and ability to determine oxygenation relationships (Davis et al., 2013; Albright et al., 2012; Davis et al., 2012). The radiologist's score (RADS) made use of chest computed tomography (CT) scans to stratify injury severity. Axial scan slices (1 cm) and radiographic findings were assessed, and the total RADS score was calculated after adding all the individually scored quadrants within the slices (Oh et al., 2012; Park et al., 2003). Based on the 0-3 RADS range, the highest score was reportedly indicative of alveolar consolidation (Oh et al., 2012; Park et al., 2003). Moreover, a significant negative correlation between RADS and PaO_2 was noted, which also potentially demonstrated the role of RADS in identifying altered oxygenation status (Megahed et al., 2023). A limitation that potentially reduced its prognostic value for injury detection during the crucial, initial injury stages was the inability to detect early injury markers such as small lesions with low density or at a cellular level (Zhan et al., 2019). Although scoring systems, particularly those for inhalation injury, were minimal, their importance was highlighted by their consistent significant correlation with prognosticated burns-related mortality.

1.4.4.2. Inhalation injury as a prognostic marker

Grading systems that prognosticated mortality were divided into two categories. One of the categories consisted of disease-related factors specific to the patients' condition, and the other comprised physiological status and medical co-morbidities for critically ill patients (Kim et al., 2019). The abbreviated burn severity index (ABSI) score was one of the most widely utilized scoring systems (El Soud et al., 2019) for the prediction of mortality likelihood (Tobiasen et al., 1982a). This model assigned respective numerical points to patients' sex, age category, inhalation injury presence, and TBSA (full

thickness) culminating in a total score ranging between 0 and 18 (Smith et al., 1994; Tobiasen et al., 1982b). A total score of 8 was estimated to have a 50% chance of mortality, and the probability of survival declined with increasing scores (Boissin et al., 2019; Dahal et al., 2016; Tobiasen et al., 1982b). Inhalation injury was assigned one point even if its presence was suspected (regardless of degree) (Boissin et al., 2019; Brusselaers et al., 2013; Tobiasen et al., 1982b), demonstrating the pivotal role the condition might play in mortality prediction, regardless of diagnostic confirmation. ABSI scores predicted survival rates that more closely resembled actual values (El Soud et al., 2019). However, it was suggested that the ABSI model was not effective in countries where resources were limited due to variations in clinical practice (Tanita et al., 2020). This prompted Yamamoto and colleagues, (2020) to modify the ABSI parameters by including the severity of inhalation injury compared to an assigned uniform score that was now based on independent measurements of associated prognostic factors (such as mechanical ventilation requirements, closed -paced injuries, bronchoscopic severity findings, etc.). It was concluded that a modified ABSI was a novel grading system that accounted for inhalation injury severity and prognosticated the associated adverse outcomes; however, its application in a clinical setting required validation (Yamamoto et al., 2020).

Other routinely used scoring systems included the Boston (Ryan) (Ryan et al., 1998), Belgium Outcome Burn Injury (BOBI) (Belgian Outcome in Burn Injury Study Group, 2009), and revised Baux (R-Baux) scores (Sheppard et al., 2011; Baux, 1961). The Boston (Ryan) score utilized a logistic regression model and three specific variables (TBSA, age, and inhalation injury) that potentiated the probability of mortality. A 0.3% chance of death was reported if all factors were absent; 3% and 33% were assigned if 1 and 2 variables were present, respectively. A score of 90% was denoted in the presence of all three factors (Ryan et al., 1998). The observed mortality, however, was reported to exceed the calculated scores (Brusselaers et al., 2005), which suggested that the model under-predicted mortality in patients with severe injuries with whom mortality was more likely to occur (Sheppard et al., 2011; Sheppard et al., 2010). The BOBI Study Group aimed to refine the Boston/Ryan score and introduced the BOBI model (Belgian Outcome in Burn Injury Study Group, 2009), which also utilized the three clinical variables. Compared to the Ryan model, a stricter definition of inhalation injury was employed, one that also required mechanical ventilation. High predictive values were demonstrated when different populations, not only Belgian, were assessed, which mitigated a common drawback observed with these systems (Brusselaers et al., 2009a; Belgian Outcome in Burn Injury Study Group, 2009). Inhalation injury was initially omitted completely from the original Baux score; however, the revised model included the parameter, now known as the R-Baux model (Osler et al., 2010). The addition of the parameter proved to be highly beneficial since the model had greater predictive value in comparison to other practical models (Woods et al., 2016; Douglas et al., 2015; Brusselaers et al., 2013).

Only R-Baux, BOBI, and ABSI scores have been externally validated (Dokter et al., 2014; Forster et al., 2011); however, additional grading systems that have shown good predictive value were the fatality

by longevity, APACHE II, measured extent of burns and sex (FLAMES) score (Gomez et al., 2008), Bull probability chart (Bull, 1971), prognostic burn index (PBI) (Yasuda et al., 1986), and Charlson co-morbidity index (CCI) (Charlson et al., 1987). All these modalities initially excluded inhalation injury in their predictive calculations, but it has since been included at later stages for some of them. The Bull's probability chart was re-evaluated to include inhalation injury and renamed the Clark's prediction model (Clark et al., 1986). The PBI system, widely used in Asian countries, also added the inhalation injury parameter to the model and noted that the mechanical ventilation aspect of the condition was important in predicting mortality (Kaita et al., 2020). The FLAMES score lacked burn-specific co-morbidities, which was indicative of its lack of predictive power (Brusselaers et al., 2009b; Tanaka et al., 2007), and it was proposed that CCI grading system be combined with the burn-specific scores to improve prediction of mortality in burn patients with burn-specific comorbidities (Knowlin et al., 2016). Unfavourable results of these scoring systems were suggested to relate to the exclusion of specific pathophysiological outcomes of cutaneous burns (i.e. ARDS, pneumonia, wound infection, sepsis, etc.) (Kim et al., 2019; Karimi et al., 2013; Sheppard et al., 2011). Overall, grading systems remain the primary burn prognostic modality, and while many are realistic enough for mortality prediction (Sheppard et al., 2011), an ideal prognostic model should be simple, reliable, objective, include burn-specific co-morbidities, and be applicable in different populations (Kim et al., 2019; Salehi et al., 2017; Tsurumi et al., 2015; Rapsang & Shyam, 2014).

1.5. Inhalation injury prognosis and diagnosis in South Africa

Very few studies have reported on inhalation injury diagnostics in SA, and even fewer have reported on inhalation injury solely, suggesting a likelihood of the condition being underreported and its prevalence potentially inaccurately reported (Whitelock-Jones et al., 1999). Of the South African studies that included inhalation injury as a potential factor, the diagnosis was based on the presence of soot in the oral or nasal cavities, dyspnea (difficulty in breathing), stridor, dysphonia (hoarseness or vocal changes), singed nasal hairs, and oro-facial burns (Cloake et al., 2017; Woodson, 2009). With the exception of the aforementioned details, numerous studies did not aim to report on the diagnosis of inhalation injury; therefore, specific details were often omitted (Boissin et al., 2019; Maritz et al., 2012). Maritz et al, (2012) stated that their related clinical parameters and surrounding circumstances were incorporated into the diagnosis of inhalation injury in their study. A few epidemiology studies have also reported on inhalation parameters recorded at TBH (in the Western Cape), which could provide a better understanding of the assessment of thermal injury severity. These studies reported on the medical records of burns patients that incorporated age (in years), gender, medical history (includes co-morbidities e.g. human immunodeficiency virus, tuberculosis, etc.), the date and time of burn injury, etiology or burn agent (hot liquid, flame, shack fire, chemical, electrical etc.), circumstances surrounding injury such as intentional or unintentional injury and alcohol intoxication at the time of injury, size (% TBSA), depth (burn degree or thickness), anatomical region or body part burnt, referral and adherence to referral criteria, hospital stay (department admitted to such as general and intensive care unit (ICU)

admission, LOS, treatment, discharge, mortality), and ABSI scores (Boissin et al., 2019; Cloake et al., 2017; Maritz et al., 2012). Some of these factors were incorporated in the criteria used for inhalation injury prognosis, i.e. the clinical index of high suspicion. More specifically, the history and physical characteristics that comprised the index included the presence of > 40% TBSA, burns on the face, upper trunk, and hands, as well as elderly or advanced age. This index, along with the ABSI scoring system, was used during the current study period at TBH's WCPATBC to determine the risk of inhalation injury and mortality, respectively.

The parameters used at TBH also overlapped with studies that reported on inhalation injury diagnosis at the Red Cross Children's Hospital (RCCH), CPT, SA (Parbhoo et al., 2010; Van Niekerk et al., 2006; Whitelock-Jones et al., 1999). Other RCCH studies addressed the presence of inhalation injury through the inclusion of history and clinical findings such as prolonged exposure in an enclosed environment, abnormal chest radiographs, decreased PaO_2, elevated COHb levels (ABG analysis), and endoscopic examination results (Rue III et al., 1993; Herndon et al., 1988; Shirani et al., 1987). FOL was also performed on children with suspected airway injury, and a modified Moylan score, which stratified inhalation injury into 3 categories: upper airway, major highway, and parenchymal injury was used (Moylan & Alexander Jr, 1978). Whether the chest radiographs and FOL were routinely used in clinical practice for diagnosis after admission was not disclosed. Collectively, these studies confirmed previous findings, demonstrating that subjective and objective measures serve as initial checkpoints for prognostication and that no standard modality has been established for diagnosis. The delay in diagnosis and interpretation of the injury in its early stages ultimately affected the survival rates of burn patients, since inhalation injury was usually only considered on admission and later confirmed using diagnostic modalities (Whitelock-Jones et al., 1999). Early diagnosis thus remained essential if morbidity and mortality rates associated with inhalation injury were to improve (Whitelock-Jones et al., 1999). Variable clinical symptomology also created diagnostic challenges, an outcome attributed to the multifarious smoke constituents (Rehberg et al., 2009) and possibly due to the differences in immunological responses between patients. Individualized treatment and clinical management of burns may be enhanced by identifying prognostic biosignatures more specific to the nature of the injury and that of the patient, which would not only predict the presence of inhalation injury, but also the severity of the pulmonary complications. This would facilitate an accurate prognosis for burn patients, especially in those with a predisposed likelihood of mortality (Finnerty et al., 2012).

2. Micro(mi)RNAs

2.1. MiRNA genomics

The human genome consists of numerous types of coding and non-coding RNA. Of the two categories, non-coding (nc)RNAs provide their function without undergoing translation into a protein (Ying et al., 2008). This group comprised the most sub-groups, *viz*. translation-related, small, and long ncRNAs,

with each sub-group further subdivided (Diamantopoulos et al., 2018). MiRNAs were first identified in 1993 as a subset of regulatory, small non-coding RNAs in the nematode *Caenorhabditis elegans* by Lee and co-workers at Harvard University. The group observed the presence of the *lin*-4 gene in the nematode, which encodes two small RNA products: a 22-nucleotide (nt) RNA and a longer 61-nt RNA. The longer RNA was predicted to have a stem-loop structure and was suggested to be the precursor of the shorter strand. The shorter *lin*-4 transcript was recognized as the first member of the miRNA family (Lee et al., 1993). These biomolecules belonged to a group of small, non-coding RNA molecules approximately 18-25 nt in length (Chou et al., 2018; Ambros, 2001; Lee et al., 1993). In humans, miRNAs accounted for 1-5% of the human genome (MacFarlane & Murphy, 2010; Rajewsky, 2006), and more than 2000 have been identified (Pan et al., 2018; Hammond, 2015; Kozomara & Griffiths-Jones, 2011). These endogenous molecules existed with numerous small RNAs, but possessed 3' hydroxyl and 5′ phosphate groups, which was a characteristic that, along with their origin and function, distinguished miRNAs from the rest of their family (MacFarlane & Murphy, 2010; Ambros et al., 2003). Initially, the miRNA precursor molecules were 60-90 nt long and presented with a hairpin, stem-loop structure as they paired with their relative target sites within one strand. After numerous processing events, the functional, single-stranded miRNA consisted of 19-25 nt (Altuvia et al., 2005; Bartel, 2004; Ambros et al., 2003). MicroRNAs were located within the host gene introns (non-coding regions) or within the intergenic regions of adjacent protein-coding sequences (Rodriguez et al., 2004; Lagos-Quintana et al., 2001). These molecules were also found in close proximity to other miRNAs on the same transcript and were presented in clusters or families. These clusters consisted of between 2 and 7 genes with short intervals that a single or numerous promoters can transcribe (Wahid et al., 2010; Kong & Han, 2005; Lagos-Quintana et al., 2001).

2.2. Biogenesis of miRNA

Prior to its mature and functional form, miRNA undergoes a series of events during biogenesis. These events were mediated by an array of proteins, which included RNA polymerase II, Drosha (an RNase III enzyme), DiGeorge Syndrome Critical Region 8 Protein (DGCR8 or Pasha, the binding partner to Drosha), Exportin-5 (XPO5, a nuclear transport receptor protein), Dicer (an RNase III enzyme), Argonaute 2 (Ago2), and RNA-induced silencing complex (RISC). The canonical (dominant) pathway follows the transcription of long primary (pri) miRNA by RNA polymerase II from different genomic locations. The priRNA was, in turn, cleaved into a hairpin precursor miRNA (pre-miRNA) by the microprocessor complex consisting of Drosha and DGCR8 (Denli et al., 2004; Han et al., 2004). The transporter, XPO5, then bound to and translocated the pre-miRNA from the nucleus into the cytoplasm, where the stem loop region was cleaved by Dicer, forming a miRNA:miRNA* (guide:passenger* strand) duplex (Okada et al., 2009; Denli et al., 2004). The duplex then consisted of a guide strand, which was the mature miRNA, and a passenger strand (Ha & Kim, 2014). The duplex then dissociates, and the passenger strand is usually degraded, where the Dicer-guide complex binds to RISC containing Ago2, forming the miRISC complex. This complex was now the mature, functional miRNA that acted on its

specific messenger (m)RNA targets (Yeung et al., 2005). Additionally, multiple non-canonical pathways were also shown to be involved in miRNA biogenesis (Hayder et al., 2018) and made use of different combinations of proteins presented in the canonical pathway comprised mainly of Drosha, Dicer, XPO5, and Ago2 (O'Brien et al., 2018).

2.3. Mechanism of action

After miRNA processing and incorporation into RISC, the mature miRNA exertes their function by interacting and regulating target mRNA in a sequence-specific manner, where the complementarity of the bases regulates the modulatory outcome (Lagos-Quintana et al., 2001). The miRNA biomolecules had a "seed sequence" (located 2–8 nt in the 5' end of mature miRNA), which was critical for target recognition, and would complementarily bind to mRNA target sites in the 3' untranslated region (UTR) of these transcripts (Bartel, 2009; Lewis et al., 2005; Lai, 2002; Ambros, 2001). Based on the amount and position of mismatching nt (complementarity within the 5' seed region and 3' part of miRNA), there were essentially 3 groups of functional target sites. One group consists of 5'-dominant canonical target sites, which paired well with both 5' and 3' ends (Brennecke et al., 2005). The second group was the 5′-dominant seed-only target sites, which were similar to the first group in function. However, it had a limited amount of base pairing with the remaining transcript, as it exhibited little or no pairing with the 3' end (Brennecke et al., 2005). The third group differed from the previous groups and consisted of mismatches at the seed site. A wide range of pairing with the miRNA 3′ region, known as the 3′ compensatory target sites, was observed (Brennecke et al., 2005). The above non-canonical pathways, however, were reported to be rare in mammals (Friedman et al., 2009). Gene expression and protein translation were thus dependent on seed sequence complementarity to its target site via two modes of binding: imperfect and perfect pairing/binding. The more common mode of binding occurred through imperfect binding, where only a specific region was perfectly complementary. This imperfect binding to partially complementary sequences led to the repression of protein translation and the sequestration of mRNA by the cytoplasmic processing bodies, which contained the mRNA degradation enzymes. Alternatively, perfect base-pairing homology resulted in RNA-mediated interference (RNAi) (Ambros, 2001), causing cleavage and degradation of mRNA by Ago2 in RISC (Van den Berg et al., 2008; Okamura et al., 2004). This perfect complementarity between miRNA and mRNA, and the resultant silencing occurred more rarely. In addition to these direct miRNA-to-mRNA binding mechanisms, recruitment of factors associated with mRNA decay, destabilization, degradation, and reduced expression levels was also stimulated and played a functional role (Bhaskaran & Mohan, 2014).

After transcript pairing, the primary ways in which gene expression and the translation of proteins were mediated by miRNA were through miRISC gene silencing, translational activation or repression, as well as transcriptional and post-transcriptional regulation (Bartel, 2004; Ambros, 2001; Lagos-Quintana et al., 2001). Gene silencing by miRISC occurs after dicer cleavage and is followed by the assembly of mature miRNA with Ago2, ultimately leading to the formation of RISC (O'Brien et al., 2018). The

Ago2 protein recruited GW182 proteins (Perconti et al., 2019), resulting in recruitment of several downstream effector proteins involved in de-capping, RNA unwinding, and deadenylation (Behm-Ansmant et al., 2006), all of which affected gene expression. Ago2 thus acted as a catalytic enzyme with the outcome of protein recruitment that promoted translational repression, mRNA destabilization (Martinez & Gregory, 2013; Iwasaki & Tomari, 2009), and ultimately targeted mRNA degradation. Moreover, translational activation was also mediated by Ago2 (in RISC) and FXR1 proteins (along with GW182) recruited into a miRNA protein complex (Bartel, 2009; Carthew & Sontheimer, 2009) that reportedly upregulated miRNA-mediated regulation (Truesdell et al., 2012; Vasudevan & Steitz, 2007) and subsequently also inhibited translation (Filipowicz et al., 2008; Peters & Meister, 2007). Translational repression was described by the Ambros group (Lee et al., 1993), which was affiliated with the discovery of the first miRNA. The group assessed nematode clones for mutants that may affect the temporal control and timing of post-embryonic development, specifically during the four different larval stages (L1-4). After the genetic screening, the presence of the *lin*-4 gene was observed, a gene crucially involved in normal temporal control in the timing of larval development. However, the gene was not found to encode for a biologically active protein but instead coded for *lin*-4 miRNA. The *lin*-4 transcripts had complementarity to repeated sequences in the 3' UTR of the *lin*-14 mRNA gene. This gene codes for the LIN-14 protein, which is abundant in the nuclei of L1 larvae and late-stage embryos. The regulation of *lin*-14 mRNA by the *lin*-4 miRNA subsequently resulted in decreased LIN-14 levels, without significant changes in *lin*-14 itself. The outcome of this repression was found to be essential in regulating the cell division transition from L1-L2 larval stages (Lee et al., 1993; Wightman et al., 1993). The study demonstrated the imperfect pairing relationship that resulted in translational repression of *lin*-14 mRNA and subsequent negatively regulated levels of LIN-14 protein.

2.4. Location and circulation of miRNA

2.4.1. MicroRNA in exosomes

Studies have reported that miRNAs are contained within small bioactive vesicles called exosomes that circulate in body fluids (Chen et al., 2008; Mitchell et al., 2008; Valadi et al., 2007) and are also bound to proteins or lipoproteins. Exosomes are membrane-bound vesicles (40-100 nm in diameter) that are actively released from various cell types, including immunocytes, platelets, endothelial, smooth muscle, and blood cells (Liao et al., 2014; Saunderson et al., 2014; Wieckowski & Whiteside, 2006). The cells released the vesicles through exocytosis and were therefore extracellularly accessible in bodily fluids (Miao, 2017; Cheng et al., 2014; Mathivanan et al., 2010). Apart from direct exocytotic shedding, cells also produce endosomes that formed smaller invaginating portions and are released as exosomes (Van der Pol et al., 2012; Gruenberg & Van der Goot, 2006; Heijnen et al., 1999). Exosomal vesicles promoted cell-cell communication, intercellular signaling, and immunoregulation by travelling varying distances in the body and transporting their regulatory contents to neighboring or distant cells (Harvey et al., 2015; Zhang et al., 2015; Valadi et al., 2007). This subsequently modulated these recipient cells (Zhang et al.,

2015; Ismail et al., 2013) and provided information regarding their functionality (Vickers & Remaley, 2012; Hong et al., 2009; Simons & Raposo, 2009). Its function, on a translational basis, was first demonstrated after the transfer of its constituents into a recipient cell (Valadi et al., 2007). However, functions were also exerted through the direct interaction between its transmembrane proteins and the signaling receptors of target cells (Munich et al., 2012). Bound forms were also released into neighboring cells after endocytosis and subsequent transcytosis, or they could undergo degradation after becoming mature lysosomes (Mulcahy et al., 2014; Tian et al., 2013). The transferred cargo determined the function which reportedly included an array of molecules, *viz.,* lipids, proteins, and various nucleic acids such as DNAs, mRNAs, and miRNAs, and other non-coding RNAs (Sato-Kuwabara et al., 2015; Moldovan et al., 2013; Simpson et al., 2012). MicroRNAs were the major constituents of exosomes and were of greater interest owing largely to their regulatory functions in gene expression and the resultant implications in both normal and diseased states (Miao, 2017; Sohel, 2016; Zhang et al., 2015). These biomarkers were estimated to regulate over a third of human protein-coding genes (Bartel, 2009) and were selectively released (Noferesti et al., 2015; Mar-Aguilar et al., 2013) by active or passive processes. An active mechanism involved release into the circulatory environment, and passive mechanisms included cell lysis, apoptosis, and necrosis (Anfossi et al., 2014; Vickers & Remaley, 2012; Arroyo et al., 2011). Moreover, the proportions and profiles in exosomes differed from those of the parent cells (Miao, 2017; Forterre et al., 2014; Goldie et al., 2014), further demonstrating their active capability in exosomes and their mechanistic roles in cells (Sohel, 2016). MiRNAs were also packed into high-density lipoproteins (Tabet et al., 2014; Vickers et al., 2011) and found externally to vesicles as Ago2-miRNA protein complexes (Arroyo et al., 2011). The locations of miRNA were ideal for stability and protection from degradation by RNases (Boon & Vickers, 2013; Vickers & Remaley, 2012; Chen et al., 2008).

2.4.2. MicroRNA in human bodily fluids

Studies have reported that miRNA exists in a stable form in numerous bodily fluids (Lv et al., 2013; Gallo et al., 2012; Zhou et al., 2012), including blood (Gallo et al., 2012; Arroyo et al., 2011; Cortez & Calin, 2009). Blood-derived serum and plasma have long been the subjects in determining which of the two materials served as a larger miRNA reservoir. Circulating miRNAs were largely evaluated in blood serum, due to its accessibility as a fresh or frozen source (Pascut et al., 2019; Schöler et al., 2011). Wang et al, (2012) demonstrated a larger yield of miRNA from serum compared to corresponding plasma samples, which was further supported by Mitchell and colleagues, (2008). The former group owed the observed difference to the coagulation process attributed to miRNA released from blood cells, as well as intrinsic and extrinsic factors (Wang et al., 2012). In contrast, Dufourd et al, (2019) demonstrated that plasma was more effective compared to serum for translational studies (Dufourd et al., 2019). Similarly, when levels of miR-15b, -16, and -24 were assessed, the plasma samples showed higher concentrations than the serum samples (McDonald et al., 2011). It was suggested that biased results between serum and plasma were attributed to the presence of cellular contaminants from various cell types such as erythrocytes or platelets; preanalytical variables such as blood tube type and storage; or

analytical variables such as differences in sample processing protocols (McDonald et al., 2011). Inconsistencies reported between plasma and serum also potentially existed due to the difference in methods used to separate the two (Tiberio et al., 2015; Cheng et al., 2013; Kroh et al., 2010).

Whole blood, as an alternative source of miRNA, had also garnered elevated interest (Patnaik et al., 2012; Häusler et al., 2010), based on the presence of various cellular sources including platelets, neutrophils, granulocytes, monocytes, red blood cells, and mononuclear cells (Atarod et al., 2014; Pritchard et al., 2012; Radom-Aizik et al., 2012) as well as the potential role of miRNAs as immunoregulatory molecules (Jasinski-Bergner et al., 2014; Okada et al., 2010; Sasaki et al., 2010). Therefore, miRNA expression levels in whole blood would be associated with both non- and blood-borne conditions (Keller et al., 2011). Studies evaluating miRNA in whole blood reported on its involvement in numerous conditions that included spontaneous preterm labour (Paquette et al., 2019); fetal hypoxia (Whitehead et al., 2013); toxicity (Correia et al., 2017; Laterza et al., 2009); pulmonary tuberculosis (Latorre et al., 2015); and pneumonia in children (Huang et al., 2018). Moreover, numerous oncological studies that utilized whole-blood samples have observed differentially expressed miRNA levels with high percentages of sensitivity and specificity (Pascut et al., 2019; Alunni-Fabbroni et al., 2018; Leidinger et al., 2014; Patnaik et al., 2012). Whole blood was also the more favoured sample type since obtaining samples only required standard blood collection, which evaded invasive approaches and was less time-consuming compared to processing required for prior separation with serum and plasma (Mariner et al., 2018; Ng et al., 2009). The differential accessibility and presence of miRNAs were closely linked to their potential as biomarkers (Molina-Pinelo et al., 2012), and based on these properties, they were suggested to be highly suitable for the prognosis and diagnosis of various pathophysiological states (De Guire et al., 2013; Jacob et al., 2013; Cortez et al., 2011).

2.5. MicroRNAs in lung development and disease

In many cell types, the common modality of miRNA modulation was the ability to form the differentiated mature state of a specific cell type from its preliminary progenitor cell type (Ivey & Srivastava, 2015). In addition, with almost all cell types, miRNAs modulated numerous cellular functions such as cell growth, proliferation, differentiation, and apoptosis (Ranganathan & Sivasankar, 2014); thus, any deviation from typical expression levels had potential adverse consequences, which was critical in pathological outcomes (Paul et al., 2018; Bhaskaran & Mohan, 2014; Tüfekci et al., 2014). The presence of miRNAs and their differential expression therefore made for useful cell recognition markers since location and function were cell and tissue-specific (Ranganathan & Sivasankar, 2014; Zhang, 2008). Its role in physiological events is reported to begin as early as embryo development and growth (Rosenbluth et al., 2014; Xie et al., 2014; Pernaute et al., 2011; Rosa et al., 2009). Specific miRNA that were upregulated in neonatal lungs were also different from those in adult lungs, illustrating the dynamic ability of various miRNAs to regulate pulmonary development during specific phases (Wang et al., 2007). Reported miRNAs involved in normal and pathological lung outcomes have

included miR-155, miR-26a, miR-29, let-7, miR-15/16, miR-233, and miR-146a/b (Tomankova et al., 2010). These were shown to play a role in lung immunity (Rodriguez et al., 2007), early lung development (Johnnidis et al., 2008; Lu et al., 2007), and lung homeostasis (Fabbri et al., 2007; Johnson et al., 2007). Moreover, miR-146a and miR-146b involvement was also observed in pulmonary inflammation and maintenance, particularly with mediating interleukin (IL)-1β, tissue necrosis factor (TNF)-α, and various proinflammatory cytokines (Abd-El-Fattah et al., 2013). Inflammatory responses to pulmonary-related viral and bacterial infiltration were also linked to the differential regulation of miR-233, miR-200a (Abd-El-Fattah et al., 2013), the miR-17 family, miR-574-5p, miR-214, and miR-98 (Mallick et al., 2009). Importantly, studies demonstrated altered miRNA profiles with respiratory pathological conditions that were dominated by inflammation such as chronic obstructive pulmonary disease (COPD) (Francis et al., 2014; Angulo et al., 2012), sarcoidosis (Jazwa et al., 2015), asthma (Wu et al., 2014), emphysema (Francis et al., 2014; Sessa & Hata, 2013), lung cancer (Huang 2013; Bianchi et al., 2011), cystic fibrosis (Viart et al., 2015; Clunes et al., 2012), ALI or ARDS (Yan et al., 2019b; Ferruelo et al., 2018; Wang et al., 2016) and tobacco/cigarette smoke related studies (Graff et al., 2012; Pottelberge et al., 2011). The ubiquitous expression of miRNAs, along with their vast modulatory functionalities, was not limited to the aforementioned findings but should provide an indication of the extent to which miRNAs can regulate tissue outcomes during normal and pathological events.

2.6. Expression of miRNAs in thermal injury

Altered miRNA levels were observed in thermal injury; however, the majority of these studies utilized rat and murine models, with limited investigations focusing on humans and inhalation injury. Denatured rat tissues and skin cells after burn-induced wounds were investigated specifically for miR-29a level because of its presence during tissue remodeling and healing. MiR-29a was up-regulated, and its inhibition increased the proliferation and migration of skin cells after injury. The potential role of miR-29a during burns and wound healing was demonstrated by its regulatory functions on skin cells, type-1-collagen and vascular endothelial growth factor-A (Zhou et al., 2016). Studies that used mouse models and excised antemortem and postmortem burned dorsal skin for analysis reported 24 differentially expressed miRNAs in both antemortem and postmortem tissues (Lyu et al., 2018). Increased levels of miR-711 and miR-183-3p were also reported in postmortem murine burnt skin and confirmed in human skin specimens (Zhang et al., 2020a). Additionally, denatured dermis (deep partial thickness burns) of burn patients was collected 4 days post-injury for miRNA analysis. A total of 66 miRNAs were identified after implementing cut-off points compared to normal skin. These profiles were confirmed by pathway analysis that showed the occurrence to be as a result of the thermal injury (Liang et al., 2012). Burns-induced conditions, such as skeletal muscle atrophy and resultant hypoglycemia, were also analyzed for differential miRNA profiles. The typical muscle wasting model of a rat tibialis anterior muscle with induced full-thickness burns for a specific period of time displayed significantly expressed miRNAs, which included miR-628 (Yu et al., 2016), rno-miR-628, and rno-miR-483-5p (Haijun et al., 2015). Pathways analysis demonstrated a direct link between its altered expression and muscle wasting

in thermal injuries (Haijun et al., 2015). Although muscle wasting typically occurs with cutaneous burns as a direct insult to the skin tissue (Walker et al., 2015; Tanizaki, 2015; Rehberg et al., 2009), similarities may exist between the two conditions due to their ability to cause injury via direct insult and subsequent inflammation. Hypoglycemia, a consequence of muscle wasting, also involved altered miRNA profiles (specifically let-7b and miR-194), and observations were confirmed using the serum of 14 burns patients (Zhang et al., 2017b). MiRNA in burn-induced outcomes after alcohol consumption have also been investigated. The expression of miR-150, in particular, was assessed in impaired intestinal epithelial cells of mice in response to ethanol consumption followed by scalding. MiR-150 modulated inflammatory mediators and was suggested to alter miRNA processing enzymes involved in biogenesis. Decreased levels of Drosher, Ago2, and miR-150 were observed, of which the latter was proposed as the agent that induced intestinal inflammation through increased IL-6 and chemokine levels. Additionally, the altered status of biogenesis components could affect the expression of numerous miRNAs involved with the condition's pathology (Morris et al., 2017).

Two reports have focused on miRNA expression profiles of inhalation injury using smoke inhalation injury-induced mice (Zhang et al., 2020c) and rat (Xiao et al., 2018) models for analysis. The latter investigated both miRNA profiles and inflammatory mediators (IL-6, IL-10, and TNF-α) in rat bronchoalveolar lavage fluid and blood plasma (Xiao et al., 2018). Zhang et al, (2020c) assessed the effects of the presence and absence of miR-155 after inducing smoke inhalation injury. Altered lung tissue damage was observed in miR-155 deficient mice, along with the reduction of neutrophil and monocyte infiltration, alveolar septum thickening, and ALI promotion. The authors reported a degree of protection against smoked-induced inflammation, which they attributed to miR-155 absence. Xiao et al, (2018) reported 25 differentially expressed miRNAs, of which 7 were validated from a group of 9 candidate miRNAs. Additionally, inflammatory mediators such as plasma IL-6, IL-10, TNF-α, and bronchoalveolar lavage fluid IL-10 increased at varying periods post-injury. These factors played a role in injury-induced inflammation typically associated with inhalation injury. Studies also observed altered IL-6 levels and miRNA expression in an inhalation injury model (Xiao et al., 2018). IL-6 and TNF-α were also shown to correlate with the severity and prognosis of inhalation injury (Ding et al., 2017; Davis et al., 2013; Finnerty et al., 2006), while IL-10 played a role as an anti-inflammatory and immunosuppressive factor (Sun et al., 2011; Wang et al., 2000). Several miRNAs were reported to regulate IL-6, -10, and TNF-α, to name a few (Salvi et al., 2019), and the association between miRNAs and cytokine regulation has been extensively reviewed (Tahamtan et al., 2018). Cytokines can be targeted directly by miRNAs or indirectly by post-transcriptionally modulating proteins that ultimately regulate cytokine levels (Salvi et al., 2019). Moreover, miRNAs were also induced by cytokines which in turn alter other cytokines e.g. IL-10 reportedly induced miR-187 with the subsequent regulation of IL-6, IL-12p40 and TNF-α (Rossato et al., 2012). Zhang et al, (2018) observed differentially expressed miRNAs that were involved with numerous inflammatory and apoptotic cellular pathways in early post-burn stages using Sprague-Dawley rat lungs with 30% induced TBSA. The bioinformatic analysis

suggested these miRNAs to be involved with numerous inflammatory and apoptotic cellular pathways in early post-burn stages (Zhang et al., 2018), which were resultant sequelae of inhalation injury (Cox et al., 2009; Gerö & Szabó, 2008). Thus, it could be postulated that should lung tissue have been analyzed in the above-mentioned reports, involvement of the same pathways may have been observed if the primary outcome was to investigate inhalation injury specifically. Active pathways in cutaneous burns may also be involved in those associated with inhalation injury; however, the complex nature of inhalation injury, the differential expression of miRNA, and the resultant involvement of additional or varying pathways could all contribute to differentiating between the two conditions.

2.7. Expression profiling of miRNAs

Determining the differential concentrations of miRNA in experimental samples has been the most ideal and straightforward method to determine interrelations with physiological processes (Várallyay et al., 2008). Numerous detection methods for miRNA profiles have been utilized and the more widely used methods include northern blotting, real-time quantitative reverse transcription polymerase chain reaction (RT-qPCR), microarray analysis, and RNA sequencing (RNA-seq) coupled with next-generation sequencing (NGS). Both mature and precursor miRNAs were detected using Northern blotting based on molecular size and abundance (Bollati & Dioni, 2019; Ye et al., 2019); however, it was only useful in detecting the expression of single, mature miRNAs (Várallyay et al., 2007). Similarly, RT-qPCR, which comprised two methods–"universal" and "specific" RT-qPCR–could only detect the expression of a single, mature miRNA. While RT-qPCR was considered the "gold standard" (Benes & Castoldi, 2010) and analyzed specific miRNAs in a sample, Northern blots assessed numerous miRNAs that were not specifically targeted (Bollati & Dioni, 2019). Albeit a highly sensitive, specific, and reproducible method for miRNA expression quantification (Shi & Chiang, 2005; Livak & Schmittgen, 2001; Heid et al., 1996), RT-qPCR was more commonly utilized for the validation of known profiles (Rooda et al., 2020; Fauth et al., 2019). Therefore, larger-scale analysis with RT-qPCR was limited by the requirement of prior analysis using high-throughput methods (*viz.* microarray and NGS analysis) to detect the expression of all nucleic profiles (Carpenter et al., 2022) before profile selection and analysis can commence. The multiplex microarray modality afforded expression analysis at a high speed (Ouyang et al., 2019) and rapidly screened numerous miRNAs using a reference ladder of known miRNA sequences. Although more cost-effective (Bollati & Dioni, 2019), the inability to detect novel sequences was a notable challenge met by the more sensitive NGS (Ouyang et al., 2019; Hunt et al., 2015; Wang & Xi, 2013).

Profiling with RNA-seq has gained popularity due to the specificity of NGS methods in transcriptome detection, i.e. miRNA-seq (Bisgin et al., 2018). Unlike the more common profiling methods, the advantage of NGS was based on its ability to profile both known, unknown, and predicted miRNAs (Stokowy et al., 2014; Liu et al., 2011); therefore, the discovery of most of the novel miRNA sequences was attributed to the employment of NGS methods (Friedländer et al., 2008). The initial steps in NGS

was to determine the miRNA sequence/s in a given sample, followed by aligning the short reads to a miRNA repository or database that contained known, available miRNA reference sequences and related data (Liu et al., 2011). RNA isolation, followed by RNA reverse transcription of the sample DNA and subsequent amplification, then took place. The latter comprised an important step called library construction that, in summary, involved adapter ligation and amplification (Hu et al., 2017; Moody et al., 2017). Adapters bind to the 5' and 3' ends of miRNA sequences and act as markers detected during amplification (Moody et al., 2017; Li et al., 2015). Emulsion or bridge PCR was utilized for amplification (Moody et al., 2017) and was repeated until complementary DNA was sufficiently produced (Ma et al., 2013) and also acted as the detected signal (Hu et al., 2017). Following successful library preparation (the completion of ligation and amplification), data and statistical analysis commenced (Li et al., 2015).

Several sequencing systems have been designed, which included Roche-454 pyrosequencing and sequencing by oligonucleotide ligation and detection (SOLiD) as well as the more commonly utilized Ion Torrent and Illumina-Solexa HiSeq/MiSeq/TruSeq systems. Roche-454 pyrosequencing and SOLiD were discontinued due to high error and cost rates, while shorter read lengths and long run times further supported the discontinuation (Saleem, 2020). The modification of Roche-454 yielded the Ion Torrent system (Saleem, 2020), which was highly advantageous in being cost-effective with less turnaround time (Kanzi et al., 2020; Diekstra et al., 2015), but not without higher false positives (Quail et al., 2012). It was, however, highly comparable (Marine et al., 2020) to the currently more popularly used Illumina NGS platform (Kanzi et al., 2020). The Illumina system allowed projects to be tailored, offering cost-effective options and design flexibility that accompanied the intended use of output data for specific study designs (Buermans & den Dunnen, 2014; Voelkerding et al., 2009). Illumina platforms also utilized sequencing-by-synthesis technology that involved bridge amplification and cluster generation on the flow cell microchip. This allowed for mass-scale, parallel sequencing of small colonies/clusters from generational copies of clonally enriched DNA templates, followed by fluorescent detection of these clusters (Shendure & Ji, 2008). Illumina-related modalities had faster turnover rates and a larger output (Liu et al., 2012), and although comparable to Ion Torrent, studies have shown favour with the former justified by lower sequencing error rates (Salipante et al., 2014; Loman et al., 2012; Quail et al., 2012; Lam et al., 2011). Additionally, Tam et al, (2014) compared a criterion of functionality on microarrays and numerous NGS systems and demonstrated the preferential use of NGS based on superior sensitivity, accuracy, and robustness for miRNA profiling in tissues. In the absence of predesigned genes/probes, NGS was the ideal candidate for miRNA expression profiling since it was not limited to only the detection and validation of known miRNAs, but also detected increased numbers and the discovery of novel miRNAs (Tam et al., 2014; Mathelier & Carbone, 2010; Motameny et al., 2010).

3. Research problem and rationale

Inhalation injury resulted in respiratory-related damage ranging from supraglottic region insult to chemical irritation and systemic toxicity (Orozco-Peláez, 2018; Sheridan, 2016; Dries & Endorf, 2013), with the latter degree representing severe clinical cases. The varying circumstances (smoke components, fire origin, exposure duration, and underlying co-morbidities (Walker et al., 2015; Rehberg et al., 2009; Traber et al., 2007) have resulted in non-specific clinical outcomes that presented with latent symptomology, manifesting up to 4 days after incidence (Masanès et al., 1995). These complexities not only made prognosis and diagnosis difficult (Sheridan, 2016), but also increased the chances of misdiagnosis (Foncerrada et al., 2018) and were associated with high morbidity and mortality rates (Lipovy et al., 2011). Since the nature of inhalation injury was complex, management and treatment had therefore largely been supportive and differed drastically from cutaneous burns, whose treatment would often only require excision and grafting (Karimi et al., 2016; Tanizaki, 2015; Dries & Endorf, 2013). While the current prognostic and diagnostic modalities remained the cornerstone of burn injury care, not many were effective enough for complete prediction (Sheppard et al., 2011). The initial diagnostic step was to evaluate the history and physical findings; however, since patients may have been unconscious and/or some of the classic signs may be absent, inhalation injury could be overlooked during this crucial period. These assessment factors alone were reportedly not strong prognostic and diagnostic indicators (Kim et al., 2017) and provided minimal insight into disease progression (Cancio, 2009). The factors have been incorporated as prognostic collectives into scoring models that have been shown to have great predictive value. However, scoring systems were reportedly too time-consuming for clinicians to make early diagnoses, and comorbidities along with applications in varying populations were often not taken into account (Kim et al., 2019; Salehi et al., 2017; Heng et al., 2015), which influenced sensitivity and specificity (Tsurumi et al., 2015). The need for prompt prognosis or early diagnosis was highlighted as pivotal and was supported by reports of a resultant reduction in morbidity and mortality (Whitelock-Jones et al., 1999).

While no standard diagnostic criteria exist, several diagnostic modalities were reported to accurately confirm the presence of inhalation injury after admission, and some have been used routinely in clinical settings. These, however, were also not without limitations which included:

(i) Impracticality involved with costs, around-the-clock trained personnel, time constraints, availability, and variability between institutions (Walker et al., 2015; Muehlberger et al., 1998).

(ii) Inability to detect injury in its initial stages and detection was confined to limited anatomical divisions, thereby displaying only a degree of injury (Muehlberger et al., 1998; Hunt et al., 1975).

(iii) Lack of sensitivity in detection or prediction (Muehlberger et al., 1998; Wittram & Kenny, 1994) and procedures may have been invasive or intolerable (Naidich et al., 1997).

(iv) Anomalies were not easily identifiable, appearing normal even 1 to 2 days post-onset (Gillen et al., 2011; Chou et al., 2004).

The above-described deficits demonstrated the necessity for a prognostic modality that would not only consider the nature of burn injuries, but would also accurately, reliably, and efficiently diagnose thermal injuries on admission or shortly thereafter. Moreover, in order to reduce associated mortality and morbidity rates, the ideal prediction model should be simple, reliable, and objective as well as have validity and the ability to be applicable within different populations (Kim et al., 2019; Salehi et al., 2017; Rapsang & Shyam, 2014). Identifying prognostic biosignatures specific to the nature of the injury and that of the patient, would allow for the prediction of the severity of pulmonary complications and subsequent mortality predisposition. This would facilitate an accurate prognosis for burn patients (Finnerty et al., 2012). Consequently, by identifying these biomarkers, medical practitioners could treat the patient in the early stages of development, even before the latent physical symptoms manifest. Ideally, exemplary biomarkers must have the following characteristics: *(i)* easily detectable and quantifiable (Pattarayan et al., 2018); *(ii)* obtainable using minimum invasive methods (Molina-Pinelo et al., 2012); *(iii)* remain stable with little to no degradation after collection (Pattarayan et al., 2018); *(iv)* well-preserved for long- and/or short-term storage, allowing for defrosting and re-cryopreservation of the samples (Liu et al., 2009a; Xi et al., 2007), and *(v)* the expression must be disease-specific (Pattarayan et al., 2018). Small non-coding sequences, i.e. miRNAs, were shown to possess all the aforementioned attributes, and their function in disease modulation has gained widespread recognition for its early predictive potential in the diagnosis and evaluation of numerous pathological diseases (Ghai & Wang, 2016; Cortez & Calin, 2009). It was postulated that these biosignatures play a crucial role in identifying and understanding the relationship between measurable biological processes and clinical outcomes, and may therefore significantly contribute to determining the presence of inhalation injury, its severity, and its potential respiratory complications.

4. Aims and objectives

The primary outcome of the study was to identify potential clinical and biological markers that could determine the presence, varying degrees and/or associated respiratory complications of inhalation injury in burn patients.

The aims and their respective objectives were:

Aim 1: To compare burns population dynamics in the current setting with those of similar demographic and/or clinical settings.
Objective: To report on the potential alignment between the sociodemographic factors, injury characteristics and clinical parameters from patients at TBH's WCPATBC with reports from similar LMIC and/or clinical backgrounds using the student-T or Mann-Whitney U test and the Fisher's exact test.

Aim 2: To assess the relationship between inhalation injury and mortality.
Objective: To evaluate inhalation injury as a potential co-factor for the mortality cases observed in burns patients admitted to TBH's WCPATBC by utilizing the Fisher's exact, Pearson's point biserial or Spearman's correlation coefficient test and partial least squares regression.

Aim 3: To analyze clinical factors contributing to the presence of inhalation injury and/or increasing degree.
Objective: To determine which clinical factors retrieved from medical files from TBH's WCPATBC and presented on or shortly after admission, *viz.* socioeconomic and injury characteristics; potentially contributed to inhalation injury presence and/or degree by utilizing the Fisher's exact, Pearson's point biserial or Spearman's correlation coefficient test and partial least squares regression.

Aim 4: To identify differentially expressed miRNA profiles from the selected burns patients admitted to TBH, WCPATBC.
Objectives:

- *(i)* To isolate total RNA samples containing miRNAs and evaluate total RNA quality (RNA integrity numbers- RIN) and quantity (small RNA percentage and absorbance readings).
- *(ii)* To identify exemplar samples based on high clinical index of suspicion, RIN values, small RNA percentage and absorbance readings.
- *(iii)* To determine differentially expressed miRNA sequences/reads of exemplar samples using Illumina NextSeq 550 platform and sRNA-bench pipeline.
- *(iv)* To identify DE miRNA for pathways analysis based on threshold cut-off values that included DE miRNAs that overlap between two sRNA-bench pipelines (EdgeR and DESeq2), has a fold difference >1.5 and Padj. value <0.05.

(v) To determine the hub DE miRNA using the miRNet online platform.

Aim 5: To determine the mRNA target genes of the differentially expressed miRNAs and identify the regulated pathological pathways potentially related to mild and severe inhalation injury.

Objectives:

(i) To identify the target mRNA target genes by utilizing the DE miRNA that met the threshold cut-off values as input in the miRNet platform.

(ii) To determine protein-protein interaction networks by utilizing the mRNA target genes as input in the STRING online platform and/or Cytoscape application.

(iii) To identify the vital module/cluster and hub target genes using the cytoHubba and MCODE Cytoscape plug-ins, respectively.

(iv) To determine the functionally enriched pathways of the hub target genes using Gene Ontology, KEGG, Reactome and PANTHER online platforms.

5. Research questions and hypotheses

Do the investigated parameters align with reports from similar demographic and/or clinical settings?

a. Null hypothesis (H_0): Parameter outcomes aligned with those observed in similar settings.
b. Alternate hypothesis (H_A): Parameter outcomes do not align with those observed in similar settings.

Did the presence of inhalation injury potentially contribute to mortality in the current burn population?

c. Null hypothesis (H_0): Inhalation injury significantly contributed to mortality.
d. Alternate hypothesis (H_A): Inhalation injury did not significantly contribute to mortality.

Did any clinical factors potentially contribute to the presence or progression of inhalation injury?

a. Null hypothesis (H_0): Specific factors significantly contributed to inhalation injury.
b. Alternate hypothesis (H_A): None of the factors significantly contributed to inhalation injury.

Were significant miRNA expression profiles detected for inhalation injury and/or varying degrees?

a. Null hypothesis (H_0): Differentially expressed miRNA profiles were identified for inhalation injury presence and varying degrees.
b. Alternate hypothesis (H_A): There were no differentially abundant miRNA profiles identified for inhalation injury presence and varying degrees.

Could differentially expressed miRNA profiles be linked to target genes and pathways potentially associated with inhalation-injury specific outcomes?

a. Null hypothesis (H_0): miRNA profiles could be linked to target genes and pathways potentially associated with inhalation injury and/or its related outcomes.

b. Alternate hypothesis (H_A): miRNA profiles could not be linked to target genes and pathways directly associated with inhalation injury and/or its related outcomes.

CHAPTER TWO
METHODOLOGY

6. Methods and materials

6.1. Ethical approval and informed patient consent

Prior to sample and data collection, the study was approved by the Health and Wellness Research Ethics Committee (HW-REC), Cape Peninsula University of Technology (CPUT), and the Stellenbosch University (SU) Health Research Ethics Committee (reference: CPUT/HW-REC 2015/H15; CPUT/HW-REC 2017/H20). Student ethics approval was also granted by the CPUT REC (CPUT/HW-REC 2017/H20). Site approval was provided from the Chief Executive Office and Medical Services Manager/Research Coordinator to conduct research at TBH, Tygerberg, CPT, WC, SA in accordance with the Provincial Policy and TBH Notice No 40/2009 (reference: CPUT/HW-REC 2015/H15). The study was also registered with the WC Government National Health Research Database (reference: 2016RP18364). All medical records and samples were obtained after patient consent, where consent forms were provided in three primary languages (English, Afrikaans, and isiXhosa). In the event that a patient could not read or understand the consent, an assigned interpreter was provided. Additionally, in the case of intubated or unconscious patients, either written or telephonic consent was attained from an immediate family member, spouse, or the medical superintendent (telephonic consent was only obtained when the immediate family member was physically absent or not reachable). Patients were also informed that they were free to withdraw their consent at any time.

6.2. Study design, setting and sample collection

This was a cross-sectional cohort study with correlational and experimental design aspects using patient medical records and blood samples, respectively. Once consent was obtained from the patient, whole blood samples and respective medical records of burn patients (N=62) were collected from the WCPATBC between 23 April 2016 and 15 August 2017 (16 months). The historical dataset serves as a critical reference point for comparative analysis over time, enabling the tracking of long-term trends, validation of findings, and assessment of shifts in disease and injury management. Aligning clinical factors, patient demographics, and prognostic indicators with existing literature would reinforce their reliability, while discrepancies may reveal evolving clinical or environmental influences. Moreover, miRNA profiles are intrinsic to biological response mechanisms and therefore retain prognostic value independent of sample collection timing and minor temporal variations in clinical practices, provided proper storage and handling. TBH was selected as a sampling site because it is the largest of two adult tertiary hospitals in the province and reportedly has the most overall fire-related fatality rates (Matzopoulos, 2005). It is the only tertiary hospital with a dedicated referral Burns Centre for adults in the Western Cape (Department of Health, 2020). The centre had a reported 22 rooms available with 16 ward beds and 6 beds in the ICU (Boissin et al., 2019; Maritz et al., 2012). Currently, the Burns Centre

comprised of 1 specialist, 1 medical officer, 2 residents from surgery, 1 intern, 40 nurses, 1 occupational therapist, and 1 physiotherapist. In the WC, for burns management, adults are classified as 13 years and older; thus, these patients are typically treated at the WCPATBC of which only 20% have been reported to fall within this category (between 13 and 20 years). Patients younger than 13 years are referred to the dedicated paediatric burns centre at the RCCH.

Medical file data of the burns patients were collected from the internal WCPATBC database and compiled by the medical officer. Only relevant, non-personal burns-related medical information was extracted from the respective patients' clinical/medical files. Patient names were not recorded; however, medical information was tracked from respective reference numbers, thus ensuring patient de-identification and anonymity in accordance with the South African Government Protection of Personal Information Act 4 (POPIA) of 2013 (South African Government, 2013). Human whole blood (2.5 ml) was collected by the assigned nurses in PAXgene® vacutainer blood RNA plastic tubes with Hemogard closure (PreAnalytix®, product code 762165). These tubes contained a stabilizing additive shown to reduce RNA degradation and minimize or eliminate gene induction in whole blood samples (Rainen et al., 2002). In addition, it was reported to stabilize the RNA quality and quantity during instant and long-term RNA preservation (Tang et al., 2019). The tubes were stored according to the manufacturer's instructions at room temperature (RT) for a minimum of 2 hrs or a maximum of 72 hrs before being stored at lower temperatures for long term-use. The sample tubes were then collected from the Burns Centre, transported on ice, and stored at -80 °C at the South African Medical Research Council, Biomedical Research and Innovation Platform for analysis. An overview of the methodological framework (initiated following ethical approval and patient consent) encompassing participant inclusion, burns-related clinical data analysis, blood sample preparation, molecular biomarker profiling, and functional interpretation, is presented in **Figure 1**.

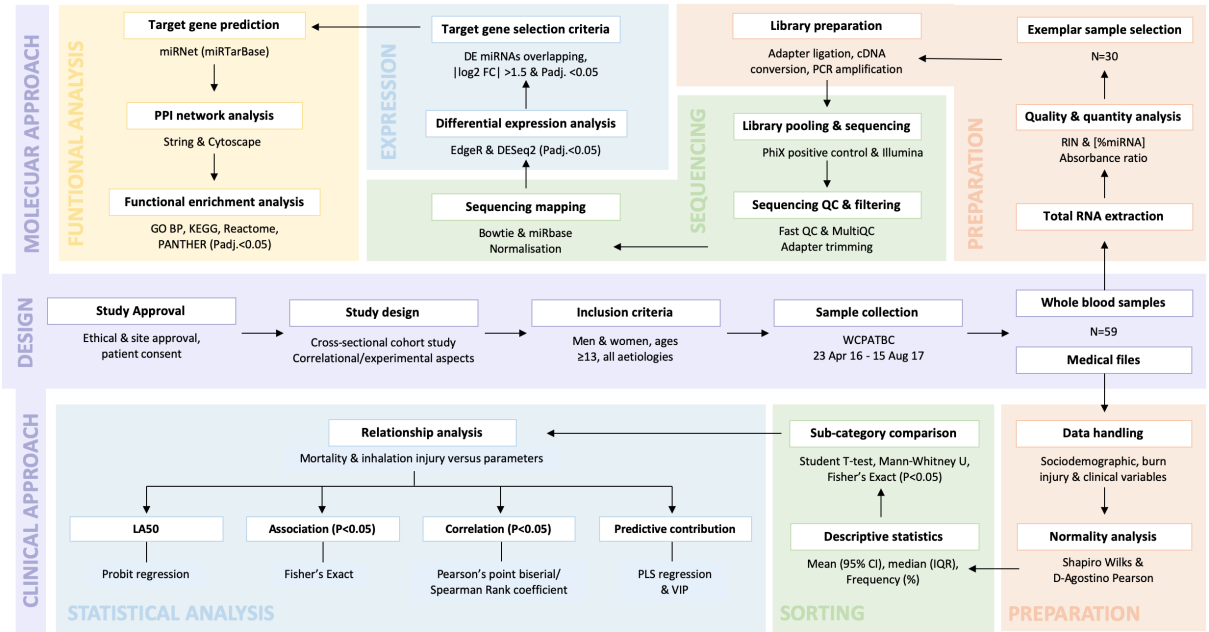


Figure 1: An overview of the methodological framework following ethical approval and patient consent: Workflow of the clinical and molecular approaches used to identify potential prognostic markers of inhalation injury in burn patients admitted to the WCPATBC between 23 April 2016 and 15 August 2017 (WCPATBC – Western Cape Provincial Adult Tertiary Burns Centre, RIN – RNA integrity numbers, QC – quality control, PCR – polymerase chain reaction, DE – differentially expressed, PPI – protein-protein interaction, Padj. – P value adjusted, GO BP – Gene Ontology biological processes, KEGG – Kyoto Encyclopedia of Genes and Genomes, CI – confidence interval, LA50 – lethal area 50, PLS – partial least squares, VIP – variable of importance in the projection).

6.3. Inclusion and exclusion criteria

The inclusion criteria incorporated men and women (prisoners, mentally challenged, aged, etc.) admitted to the WCPATBC and all patients aged ≥ 13 years that were treated as adults independent of the type of burns sustained. The exclusion criteria included patients under 13 years, patients re-admitted who had not been sampled with initial admittance, and patients with cardiac-related pulmonary edema and hypoxemia.

6.4. Data sorting and stratification

Patient information was grouped within the following subcategories: demographics, injury characteristics, and clinical parameters. Each subcategory had the following respective subgroups, for demographic factors: gender (male and female), age groups (13-20; 21-39; 40-49 and ≥50 years), day of the week (week and weekend days), seasonal variation (colder and warmer seasons), referral setting according to WC district divisions, level of referring hospital (hospital and clinic or community health centre), and days between injury incidence and admission (0-2; 3-5 and >5 days). Injury characteristics included aetiology (fire and other), %TBSA (≤40% and >40%), inhalation injury (mild injury based on 0-4 days total days ventilation and severe injury denoted by ≥ 5 days), phenotypical characteristics (presence or absence of singed nasal hairs, soot around or in the mouth, hoarseness and facial burns), complications (yes and no), and ABSI scores (moderate 4-5, moderately severe 6-7, serious 8-9, severe 10-11, and maximum 12-13). The Lethal Area fifty (LA50), indicative of the percentage of TBSA burns expected to result in 50% of burns-related deaths (Bull & Squire, 1949), was determined using Probit regression analysis of survivability by TBSA. Clinical parameters included LOS in burns intensive care unit (BICU) (0-9 and ≥10 days), ventilation prior to admission (yes and no) and arterial blood gas analysis i.e. blood pH (<7.35; 7.35-7.45 and >7.45), PaO_2 (<10.5; 10.5-13.5 and >13.5 kPa), PCO_2 (<4.7; 4.7-6.0 and >6.0 kPa), SATS (<95 and 95-100%), lactate levels (<2.0 and ≥2.0 mmol/L), BE levels (<-4; -4 to +2 and >2 mmol/L), and mortality (yes and no). Continuous variables included age, days between injury incidence and admission, %TBSA, inhalation injury (total days of ventilation), ABSI scores, BICU LOS, and all blood gas parameters. These were coded accordingly for analysis that required categorical variables.

6.5. Data analysis

6.5.1. Descriptive statistics and association

Data was captured and collated using Microsoft Excel 2016 version 16.16.7 (Microsoft Office, Redmond, WA, USA) and checked for completeness and consistency prior to statistical analysis. Since analysis predominantly required paired observations, 3 patients were excluded due to omission of information (N=59). All statistical analysis of the data was performed using the Statistical Packages for the Social Sciences program version 23 (IBM SPSS Inc., Chicago, IL, USA). Figures and tables were generated using Microsoft Word 2016. Descriptive data were reported as incidence, frequency, and proportions [patients, *N* (%)] for categorical variables. For continuous data, central indices were reported as mean and 95% confidence interval (95% CI) for normally distributed data or median and interquartile range (IQR) for non-normally distributed data. Tests for normality included the D'Agostino Pearson and Shapiro-Wilk's normality tests. The two-tailed, unpaired T-test and appropriate analysis of variance (ANOVA) was used to compare two or more means of normally distributed data with the Tukey's test as a post hoc test for comparison. When the residuals were non-normally distributed, the Mann-Whitney U test or Kruskal-Wallis test was conducted to compare two or more medians of categories, and the Dunn's test was used as a post hoc test for comparison. The Fisher's exact test was used for the association between mortality, inhalation injury, and the selected variables. The latter test required categorical variables; therefore, the continuous variables were coded into respective subgroups. All levels of statistical significance for all statistical tests were two-tailed, and significance was set at $P<0.05$.

6.5.2. Correlation coefficient analysis

For significant correlations between variables, the Pearson's point-biserial correlation coefficient (r_{pb}) or Spearman's rank correlation co-efficient (rho) with a 95% CI was selected based on the statistical assumptions being met or violated. The correlation coefficients estimated the strength and direction between the dependent variable (DV) and independent variable (IV), which in turn, was based on the value being positive or negative (direction) and the value's allocated range of correlation (size or strength). DVs were indicative of the outcomes or conditions, i.e. mortality and inhalation injury, while IVs represented the demographical, burn injury, and clinical parameters. The Spearman's rho demonstrated no level of correlation if the coefficient was between 0.01 and 0.19, while 0.20 to 0.29 indicated weak correlation, 0.30 to 0.39 had moderate correlation, 0.40-0.69 had strong correlation, and a very strong correlation was observed with rho values ≥0.70 (Dancey & Reidy, 2007). The Pearson's r_{pb} illustrated a very weak correlation for a 0.00 to 0.19-ranged value, a weak correlation for 0.20 to 0.39, 0.40 to 0.59 for moderate, 0.60-0.79 for strong, and very strong correlations were denoted with the 0.80 to 1.00 range (LeBlanc & Cox, 2017; Meghanathan, 2016; Mukaka, 2012). With both tests, a positive coefficient indicated a direct correlation, i.e. when the IV increased or was present, so did the

DV, and vice versa. A negative coefficient indicated an opposing correlation, i.e. when the IV increased or was present, the DV outcome would be the inverse.

6.5.3. Partial least squares regression

Partial least squares (PLS) regression was used to determine which significantly correlated explanatory independent X-variables (IVs) best predicted the response dependent Y-variables (DVs) (Wold, 1985). The selection was based on the data set being particularly smaller compared to the requirements for the traditionally used regression models and was suitable for data with multicollinearity or complete/quasi complete separation. The model was based on the formation of latent variables from linear combinations of the original variables. The latent structure of X that described the most variation in Y (highest adjusted R-square value) depicted the best predictive model and corresponding variable of importance in the projection (VIP) values were generated. VIP values, therefore, provided an estimation of the X-variables' contribution to Y, and the predictor variables with VIP scores >1 indicated a strong contribution, which was most relevant for explaining the outcome (Akarachantachote et al., 2014; Eriksson et al., 2006; Wold, 1985). Values of >0.5 were previously reported as significant (Ramabulana et al., 2020) and also considered in the current study. The variables were initially utilized in their original data type in order to determine which had significant VIP scores (>0.5). Once identified, the model reintroduced the subgroups of the continuous variables to determine the specific subgroup(s) that contributed more to the DV. Two separate models were generated (one per DV), and latent factor selection with adjusted R-square (R^2) values and respective VIP values were reported. The remaining lower-order latent variables were associated with noise in the process and therefore not considered (Li et al., 2001).

6.6. RNA sequencing and analysis

6.6.1. Total RNA isolation

Buffers and working solutions for all included kits were reconstituted and stored according to the manufacturer's instructions. Buffers, samples, and working solutions were thawed, allowed to calibrate to RT, or placed on ice depending on the assessment specifications. In order to avoid RNase activity, surfaces were wiped down using RNase-free water and RNaseZAP wipes. Total RNA extraction was performed using the PAXgene® blood miRNA kit (PreAnalytix®, product code 763134), and the protocol was followed according to the manufacturer's instructions. Using the kit, samples underwent a series of washes with buffers and DNase treatment, and once the final sample elute in buffer (BR5) was obtained, it was immediately placed on ice for quantification followed by storage at -80 °C for quality assessment. Treatment with DNase ensured that DNA-related products would be digested, allowing RNA products to remain intact and present.

6.6.2. RNA quantity and quality analysis

Total RNA concentration was quantified using the Nanodrop ND-100 (Nanodrop Technologies) and absorbance measurements. The absorbance ratio at 260/280 nm (used as an RNA purity marker) was measured in triplicate. For gene expression, the acceptable optical density-$OD_{260/280}$ ratio was approximately 2.0, but the ratio of 1.8 and above was also deemed suitable (Desjardins & Conklin, 2011; Nybo, 2011). The quality analysis was performed by the Central Analytical Facilities, SU, using the Agilent 2100 Bioanalyzer (Agilent Technologies), which implemented a lab-on-chip microfluidics system via electrophoresis (Fleige & Pfaffl, 2006; Schroeder et al., 2006; Mueller, 2004). The RNA 6000 Nano kit was used (Agilent Technologies, product code 5067-1511) and allowed for the standardization of RNA quality control and generated RNA integrity numbers (RIN). A RIN of 1 indicated complete sample degradation, and 10 indicated an intact RNA sample (Fleige & Pfaffl, 2006; Schroeder et al., 2006; Mueller, 2004). The small RNA kit (Agilent Technologies, product code 5067-1548) was used to determine miRNA concentration (%) based on the detection of particles within the 10-40 nt size range, which was then quantified as absolute amounts [pg/µl] or as a fraction [%] relative to the total small RNA (Peiró-Chova et al., 2013; Becker et al., 2010).

6.6.3. Exemplar samples selection criteria for sequencing

Thirty (30) exemplar samples were selected based on sequencing prerequisites, their high clinical index of suspicion, and additional clinical parameters utilized by the WCPATBC. These parameters included: *(1)* quantity of 2-10 µg total RNA per sample, *(2)* an A260/280 ratio of between 1.8 and 2.0 and an A260/230 ratio of between 1.8 and 2.0, *(3)* quality values of RIN > 7 (Puchta et al., 2020; Sheng et al., 2016), *(4)* the presence of associated phenotypical characteristics such nasal hair singing, soot around and/or in the mouth, hoarseness, facial and hand burns, *(5)* mortality, *(6)* fire etiology (predominantly), and *(8)* total ventilation days (the longer the days on ventilation, the stronger the likelihood of injury presence, i.e. ≤ 4 days = mild injury). All of these factors indicated efficient sample quantity and quality for sequencing, while the parameters of high clinical index of suspicion and selected clinical factors determined the strong likelihood of injury presence and varying degrees.

6.6.4. Small RNA-seq library preparation and quality control

Differential abundance analysis on exemplar sample (N=30) aliquots (2-10 µg/20 µl total RNA in BR5 buffer) was performed at the Centre for Proteomic and Genetic Research using NGS, and specifically the Illumina NextSeq 500/50 sequencing platform. The samples represented two degrees of inhalation injury, i.e. mild (N=15) and severe (N=15). The total RNA library (200 ng per sample) was prepared using the NEXTFLEX small RNA-seq kit v3 (Perkin Elmer®, #NOVA-5132-05) according to the manufacturer's instructions and included the following steps: *(1)* NEXTFLEX® 3' 4N adenylated adapter ligation, *(2)* excess 3' adapter removal and adapter inactivation, *(3)* NEXTFLEX® 5' 4N adapter ligation, *(4)* reverse transcription-first strand synthesis and bead clean-up, *(5)* PCR amplification and primer (universal and barcoded) incorporation, and *(6)* gel-free size selection & clean-up. The average

size distribution for each library was then determined using the TapeStation D1000 High Sensitivity ScreenTape assay (Agilent Technologies, product code NC1786959) as per the manufacturer's instructions. Following appropriate preparation with the assay, the samples were analyzed with the Agilent 4200 TapeStation instrument (Agilent Technologies), and electropherograms were generated. Upper and lower reference markers (size standards) were represented by peaks at 25 and 1500 bp, respectively. Average fragment size distribution was denoted by an expected peak or smear between 150 and 200 bp based on TapeStation assessment. An additional gel-free size selection & clean-up was carried out on all samples to remove the additional impurities or contaminants. The purified small RNA-seq libraries were quantified using the Qubit 1X dsDNA HS Assay Kit (Invitrogen™, product code Q33230) according to the manufacturer's instructions, and the resultant fluorescence was measured using Qubit Fluorometer (Invitrogen™ Qubit™). Concentration was calculated using the following formula:

$$\text{Concentration [ng/ml]} = \frac{\text{Purified small RNA library absorbance reading (nm) X 200}}{\text{Volume fo sample in tube (ml)}}$$

The assay was recommended for initial sample concentrations ranging from 10 pg/µl to 100 ng/µl, which delivered more accurate detection and provided a core detection range of 0.2 ng to 100 ng of DNA.

6.6.5. Small RNA-seq library pooling and sequencing

The small RNA-seq libraries were pooled for sequencing with each library diluted to 2 nM, and each library was added to the final sequencing library pool. The average fragment size for the pooled library was determined using the TapeStation D1000 High Sensitivity ScreenTape assay according to the manufacturer's instructions (as previously described). Prior to sequencing, the NextSeq 500/550 High Output kit v2 (75 cycles) (Illumina, product code 20024906) was used as per the manufacturer's instructions for library preparation, which also included the necessary cartridges and reagents. The pooled library was denatured with 0.2 N sodium hydroxide and diluted to 1.8 pM. The denatured library was then combined with the diluted PhiX positive control (sequencing control) at a spike-in concentration of 1% v/v according to the specific Illumina protocol. The prepared pooled library was loaded (1.3 ml) into the designated reservoir of the reagent cartridge, which, along with the flow cell and buffer cartridge, was loaded into the Illumina NextSeq 500/550 cycle instrument (Illumina). After initiation of the sequence run, libraries were transferred automatically from the reservoir to the flow cell. The sequencer was programmed to perform a single-end, single-indexed 1x51cycle sequencing run. FastQ files at the end of the run and a summary of quality metrics after sequencing was generated.

6.6.6. Sequencing pre-quality and quality control

Pre-quality control and quality control were performed on the raw FastQ files, which assessed the quality and length of reads as well as determined the presence of potential adapter content. Before mapping, Fast QC (Andrews, 2010) was used to assess the quality of the sequence data, and a quality report was

generated for each sample, which was aggregated into a single report using MultiQC (Ewels et al., 2016). The quality analysis was based on the key performance metrics, including per base sequence quality, per sequence GC content, sequence duplication, and over-representation. MultiQC produced colour-coded QC figures as output; red was indicative of "failed", orange "warning", and green "pass". As recommended, the default settings were assumed in both the MultiQC and FastQC programs. The sRNA-bench toolbox (Aparicio-Puerta et al., 2022) pipeline was then used to trim any adapter content, and only sequences that contained at least 10 nt were retained for downstream analysis. Low-quality reads with less than 20 Phred scores were filtered out from the downstream analysis.

6.6.7. Differential expression analysis of miRNAs

For mapping and subsequent miRNA identification, the genome mode of the sRNA bench pipeline was applied. The processed reads were then aligned to the human genome GRCh38 with Bowtie, allowing zero mismatch of the aligned reads coordinates. The reads were then also subjected to the miRBase database v22 (human reference sequences), and those aligning to miRNA sequences were then quantified with the sRNAde program. The latter was implemented for generating count data from the aligned files, which was normalized as counts per million input prior to differential expression (DE) analysis. EdgeR (Robinson & Oshlack, 2010) in R statistical software v4.1.2 (R Core Team, 2011), which in turn used the trimmed mean of M-values for normalizing differences across RNA composition, was used for DE analysis. Fisher's Exact test was used to compare mild and severe groups' miRNA. P-adjusted (Padj.)<0.05 values denoted differential miRNA expression, which were values corrected for multiple testing with the Benjamini-Hochberg false discovery rate (FDR) method (Storey & Tibshirani, 2003). DESeq2 was used as an additional tool to corroborate overlapping DE miRNAs. Microsoft Excel 2016 version 16.16.7 (Microsoft Office, Redmond, WA, USA), heatmaps were generated with the online SRPlot program (Tang et al., 2023), and volcano and violin box plots were compiled with GraphPad Prism version 8.4.2 (GraphPad Software., Boston, MA, USA).

6.6.8. Target gene analysis of differentially expressed miRNAs

The following thresholds were used to select the DE miRNAs for target gene analysis: *(1)* DE miRNAs that overlapped with EdgeR and DESeq2, *(2)* with expression value fold changes (FC) |log2 FC|>1.5 and *(3)* Padj.<0.05 (Song et al., 2022; Robinson et al., 2010; Mestdagh et al., 2009). In order to observe the miRNA-mRNA network, the downstream target genes of the selected DE miRNA were predicted with the miRNet platform, version 2.0 (http://www.mirnet.ca/), and the network interaction data was collected using the mirBase IDs and miRTarBase, Version 9.0, that returns results based on experimentally validated findings (Huang et al., 2022a). The input of up- and down-regulated DE miRNAs as well as both groups combined were included in the analysis, and the resultant networks were visualized with the topology graph feature (default layout and/or force atlas). The integrated topology features were reported, which included degree centrality, which represented the number of connections

(edges) to other nodes, and betweenness centrality, which measured the number of shortest paths going through a node.

6.6.9. Construction of protein-protein interaction network, clusters, and hub genes

Protein–protein interaction (PPI) networks have been important in deciphering connections in network structures and biological function (Yook et al., 2004) due to the interactive roles of proteins in molecular and cellular mechanisms that control physiological and pathological conditions (Safari-Alighiarloo et al., 2014). Therefore, the PPI network was constructed by importing the identified target genes of miRNET into the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) version 12.0 (http://string-db.org). STRING retrieves and displays repeatedly occurring gene neighbourhoods (Szklarczyk et al., 2019; Szklarczyk et al., 2015; Snel, 2000), which were based on the combined interaction score between the gene (node) pairs of >0.4 (medium confidence) and Padj.<0.05 (Zhou et al., 2024; Rezaeijo et al., 2023; Li et al., 2018). The PPI network was then imported directly into Cytoscape 3.10.3 (https://cytoscape.org/) (Shannon et al., 2003) to visually transform the results and for further analysis with the platform's plugins. The StringApp, version 2.1.1 plug-in, was used to generate the PPI network if the target gene input was ≥ 2000). The molecular complex detection (MCODE) plug-in was applied to determine the most significant core module (cluster) of the PPI network with the following parameter thresholds: MCODE scores >5, degree = 2, node score = 0.2, k-score = 2. and max depth = 100 (Zhai et al., 2022; Li et al., 2018; Bader & Hogue, 2003). The highest MCODE score denoted the most significant cluster in the network. Using the clusters, the cytoHubba network analyzer plug-in was then used to determine the hub genes of the PPIs through connectivity degree, and the top 10 hub genes were presented per network (Wang et al., 2024; Liu et al., 2020).

6.6.10. Functional annotation and pathway enrichment analysis

The top 10 hub genes of each target gene network (up-, down-regulated, and the combined) were subjected to functional enrichment pathway analysis to assess potential biological functions and associated pathways (Dennis et al., 2003). Gene ontology (GO) biological processes (BP) terms, Kyoto Encyclopedia of Genes and Genomes (KEGG), Reactome, and PANTHER pathways were retrieved from the online EnrichR platform (Xie et al., 2021; Kuleshov et al., 2016; Chen et al., 2013a). GO biological process-enriched terms were focused on (Carbon et al., 2021; Ashburner et al., 2000) along with KEGG, which comprised detailed metabolic pathways (Kanehisa et al., 2017; Goto et al., 1997) and Reactome (Joshi-Tope, 2005) and PANTHER (Mi et al., 2012; Mi & Thomas, 2009) both of which curated human pathways; however, the latter was particular for those involved with signaling. Hypergeometric distribution was utilized for significance calculation (Rivals et al., 2007), and the FDR-corrected P-value (Padj.)<0.05 was considered notable. The top 10 enriched outputs were presented by vertical bar graphs retrieved and modified from EnrichR.

CHAPTER THREE
EPIDEMIOLOGY AND BURNS OUTCOMES

7. Epidemiology of burns patients admitted to the WCPATBC

7.1. Overview

Burn injuries have remained a significant public health concern despite improved injury care and awareness (Ahn & Maitz, 2012). A large proportion of thermal incidences reportedly occurred in LMICs, with WHO-Asian and African regions frequently associated with mortality (World Health Organization, 2018a). In SA, 3.2% of the population suffered from thermal injury annually (Allorto et al., 2016; Rode et al., 2014), and the outcomes were influenced by numerous factors, from socio-demographic circumstances to the nature and characteristics of the injury itself. Contrary to reports from the paediatric cohort, the clinical course and outcomes of adult burn patients were limited. Within the socio-economic South African context, data presenting factors that drive, and influence burn-related incidences were lacking, and while this information was readily available in international databases, the current, limited local repositories need enhancement so that preventative and treatment measures could be proposed. Therefore, the objectives of this chapter were to *(a)* provide a comprehensive descriptive report of the hospitalized burns population and mortality outcomes at TBH's WCPATBC that is otherwise limited in the available literature, and *(b)* determine trends in the admission parameters in similar clinical settings. The parameters recorded on admission or shortly thereafter included the following: *(i)* sociodemographic factors *viz*. gender, age, day on which the incidence occurred, the seasonal variation of the incidence, the district the patient was referred from, and the clinical level of referral; *(ii)* injury characteristics *viz*. aetiology, %TBSA, inhalation injury degree, phenotypical characteristics, burns-related complications, and ABSI scores; and *(iii)* clinical parameters *viz*. BICU LOS, ventilation before admission, and ABG measurements (pH, PaO_2, PCO_2, Sats, lactate, and BE levels). The Student T-, Mann-Whitney U, and/or Fisher's Exact tests were used (see Chapter 2: Methodology) for comparison within subgroups, which was dependent on the nature of the data. Central indices were reported accordingly, and mortality was described as both incidence (counts) and as rate (a percentage of respective total admissions).

7.2. Results

7.2.1. Sociodemographic, injury and clinical factors of burns patients admitted to the WCPATBC

Male patients attributed to nearly two thirds (64.4%; p=0.000) of the study population (n=59), with a male-to-female ratio of 1.81:1. Patient age ranged from 15 to 55 years with a mean of 32.8 years (CI=30.0-35.6), and thus majority of admissions were observed within the 21-39 year age group (64.4%; p=0.000). Temporal parameters revealed injury predominance on weekdays (62.7%; p=0.000) and in

the colder seasons (p=0.000). Significant admissions were also observed with patients referred from the City of Cape Town district (84.7%; p=0.000) and from hospital settings (78%; p=0.000) (**Figure 2, Appendix A: Table 9)**.

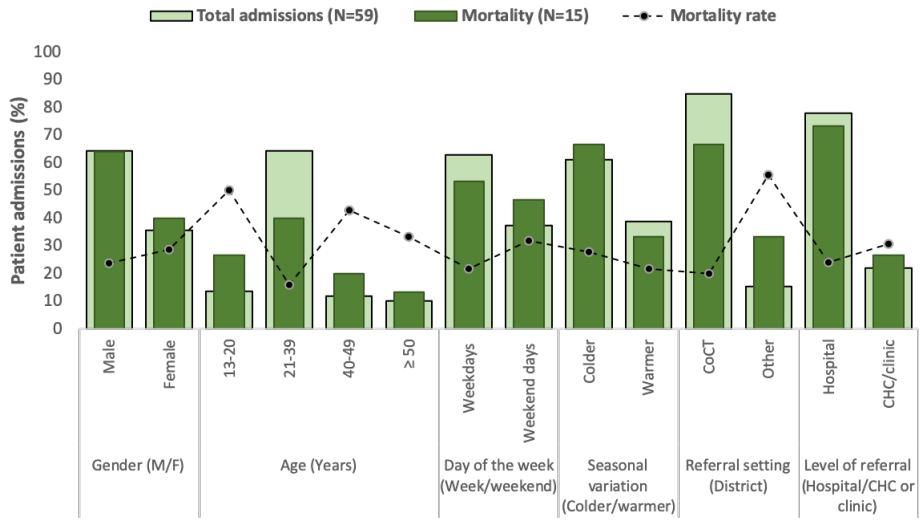


Figure 2: Sociodemographic factors and mortality outcomes: Burns-related sociodemographic factors for the burns patients admitted to (N=59) and the mortality cases (N=15) at the WCPATBC during the 18-month period (M – Male, F – Female, CoCT – City of Cape Town, CHC – community health centres).

Most of the burn injuries were sustained by flame burns (74.6%; p=0.000) and approximately 88% of patients had %TBSA less than 40% (88.1%; p=0.000) averaging at 23.4% (CI=19.4-27.3). Patients were more frequented with facial burns (88.1%; p=0.000) followed by soot around the mouth and nasal hair singing (67.8% and 66.1%, respectively). A significant number of patients also had severe inhalation injury (61.0%; p=0.000) and were ventilated for 5.5 median total days (IQR=4.0-12.0). Complications presented in less than half of the population (42.4%) and included septic shock, septicaemia, ARDS, aspiration pneumonia and VAP. ABSI scores indicated that the larger patient groups (n=40; $p \leq 0.020$) scored between 6 and 9 representing moderately severe to serious outcomes with a mean ABSI score of 8.0 (IQR=6.0-9.0) (**Figure 3, Appendix A: Table 10)**.

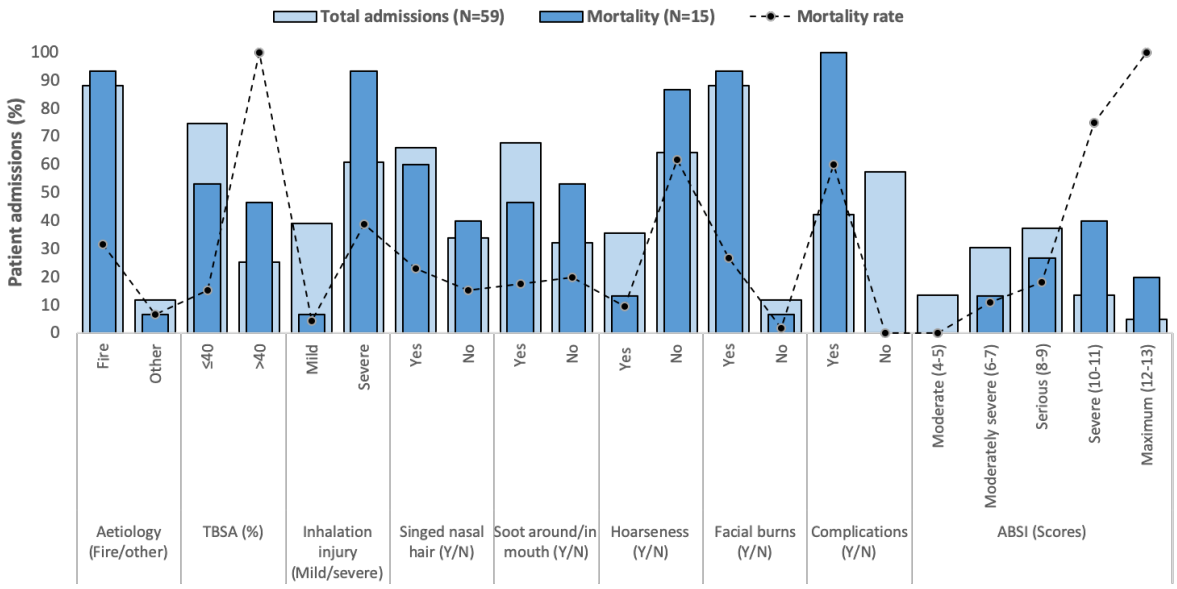


Figure 3: Burn injury factors and mortality outcomes: Burns injury-related factors for the burns patients admitted to (N=59) and the mortality cases (N=15) at the WCPATBC during the 18-month period (TBSA – total body surface area, Y – yes, N – no, ABSI – abbreviated burn severity index).

Approximately 51% had a BICU LOS of 0-9 days (p=0.000) with a median stay of 9.0 days (IQR=5.0-14.0), and at least 86% of patients were ventilated prior to admission (p=0.000). Patients presented with the following mean and median ABG measurements: pH of 7.36 (CI=7.34-7.38), PaO_2 of 18.6 kPa (CI=16.7-20.6), PCO_2 of 5.4 kPa (CI=5.1-5.8), Sats of 99.0% (IQR=97.0-100.0), lactate levels of 1.7 mmol/L (IQR=1.2-2.4 CI) and base excess of -1.8 mmol/L (IQR=-3.7-0.0). Majority of patients had ABG measurements within the normal ranges which included: pH (47.5%; p≤0.011), PCO_2 (45.2%; p=0A.000), Sats (88.1%; p=0.000) and base excess (69.5%; p=0.000). In addition, 69.5% patients had excess PaO_2 (p=0.000) and 57.6% had reduced lactate (p≤0.000) (**Figure 4, Appendix A: Table 11)**.

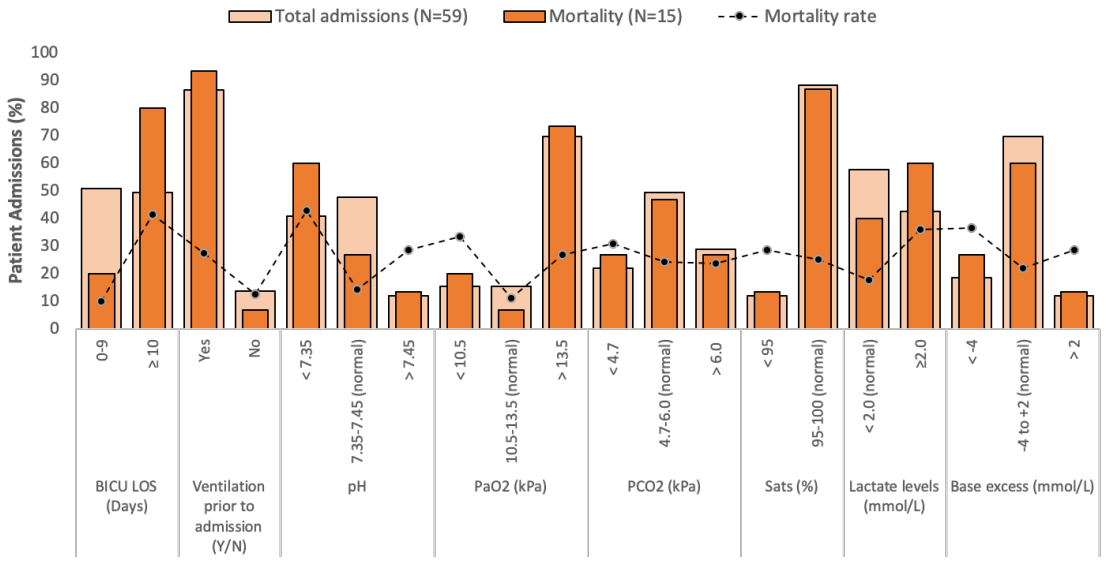


Figure 4: Clinical factors and mortality outcomes: Burns-related clinical factors for the burns patients admitted to (N=59) and the mortality cases (N=15) at the WCPATBC during the 18-month period (BICU LOS – burns

intensive care unit length of stay, Y – yes, N – no, PaO_2 – oxygen partial pressure , kPa – kilopascal, PCO_2 – carbon dioxide partial pressure).

7.2.2. Sociodemographic, injury and clinical factors of deceased burns patients at the WCPATBC

Elevated mortality counts were noted with the following subgroups that also displayed higher numbers in admissions: male patients, 21-39-year age group (mean=34.3 years; CI=26.9-41.6), weekdays, colder seasons, CoCT referral setting and hospital referral level, fire aetiology, ≤40% TBSA (mean=37.5%, CI=29.4-45.7), severe inhalation injury (mean=11.8 total days ventilated; CI=9.3-14.3), the presence of most of the phenotypical characteristics (i.e. facial burns, singed nasal hair and soot in/around the mouth), ventilation prior to admission, excess PaO_2 (mean=20.1 kPa; CI=14.6-25.5), normal PCO_2 (mean=5.2 kPa; CI=4.5-5.9), SATS (mean=97.9%; CI=96.4-99.5) and BE (mean=-2.3 mmol/L; CI=-6.2-1.6) levels. Higher mortality rates were also associated with some of the afore-mentioned factors *viz*. colder seasons (27.8%), fire aetiology (31.8%), severe inhalation injury (38.9%), the phenotypical characteristics and ventilation prior to admission (27.4%) (**Figures 2-4, Appendix A: Tables 9-11)**.

Higher mortality incidences noted within groups that had less admissions included patients with the presence of complications, ABSI scores of 10-11 (mean=9.7 score; CI=8.7-10.8), longer BICU LOS (mean=13.4 days; CI=10.5-16.3), acidotic pH (mean=7.33 (CI=7.28-7.38) and excess lactate levels (mean=3.1 mmol/L (CI=1.6-4.7) (**Figures 3-4, Appendix A: Table 10 & 11)**. Elevated mortality rates were observed for all of these variables in addition to some with lower mortality counts. A predominant rate was noted among the female population (28.6%) and an age-related preponderance was attributed to the youngest (50%) and older grouped patients (≤33.3%). Temporal parameters associated with the mortality rate were linked to weekends (31.8%) in addition to patients referred from CHC/clinics (30.8%) and areas outside the CoCT (55.6%) (**Figure 2, Appendix A: Table 9)** Patients with >40% TBSA were linked to 100% death rate and 60% were related to complications. Severe (10-11) and maximum (12-13) ABSI scores were attributed to a cumulative of 9 deceased patients and were affiliated with the highest mortality rates (75% for severe and 100% for maximum) (**Figure 3, Appendix A: Table 10)**. The BICU LOS for 12 of the 15 mortality cases was <10 days with an elevated mortality rate of 41.4% for patients in this group. Nearly all clinical parameters with ranges below normal levels significantly related to increased mortality rates, with the exception of excess lactate levels (36%) (**Figure 4, Appendix A: Table 11)**.

7.3. Discussion

In the current clinical settings, increased prevalence in the admissions of burn patients was observed with the male cohort and the 21-39-year age group. This trend was also observed with previous burn patterns reported in SA and other LMICs (Keyes & Liphoko, 2021; Cloake et al., 2017; Maritz et al., 2012; Van Niekerk et al., 2009; Eyal et al., 2007; Godwin et al., 1997) and with a comparable average

age range of 30-34.3 years (Boissin et al., 2019; Daffue et al., 2018; Den Hollander et al., 2014; Gevaart-Durkin et al., 2014; Maritz et al., 2012; Godwin & Wood, 1998; Godwin et al., 1997). Previous studies also reported higher incidences with males in this age group (Keyes & Liphoko, 2021; Boissin et al., 2019; Blom et al., 2016). These age differences provided insight into the demographics of the burns' population admitted to TBH's WCPATBC. Since younger adults predominantly comprise the highly populated, informal dwelling environments, this group may contribute significantly to the impact that this burden of disease has on developing countries (Eyal et al., 2007). It was indeed reported that this age group constituted a large proportion of the reported working population (15-64 years) in SA (Statistics South Africa, 2020; City of Cape Town, 2016; Hunter & Posel, 2012) with the average South African working age reportedly being 32.75 years within the informal dwelling settings (Hunter & Posel, 2012). The frequency of burn injuries within this age group could further exacerbate socio-economic conditions (Eyal et al., 2007). Causative factors have been affiliated with low-income circumstances, some of which were to the detriment of males and the 21-39 age group. Elevated burns-related violence and assault by means of fire and scalding have been observed in both the gender sub-group and the younger age group (Cloake et al., 2017; Blom et al., 2016; Gevaart-Durkin et al., 2014; Theodorou et al., 2009) and were also linked to increased reports of suspected substance (alcohol and other) over-consumption (Blom et al., 2016; Maritz et al., 2012; Van Niekerk et al., 2009). Furthermore, the nature of work engagement or employment was also highlighted as a contributing factor (Ringo & Chilonga, 2014; Blom et al., 2011; Edelman et al., 2007), for males who in particular were exposed to high risk environments *viz*. mines and burning fields (Blom et al., 2011). Other factors proposed to be linked to male-and/or adolescent group-related thermal incidences include increased impulsive and risk-forward learnt behaviour (Rosen & Peterson, 1990; Eaton & Yu, 1989), cable theft in cases of electrocution (Blumenthal, 2009); improper use of flammable substances (Forjuoh, 2006), self-inflicted injuries (Cloake et al., 2017; Gevaart-Durkin et al., 2014), and housing and education (Ringo & Chilonga, 2014; Edelman et al., 2007; Godwin & Wood, 1998). Contrasting studies have reported an injury preponderance in females (Sivamuthu, 2019; Davé et al., 2018; Hemeda et al., 2003), while a near equal or no clear differences have also been observed (Agbenorku et al., 2019; Blom et al., 2016; Rode et al., 2015).

Females, patients younger than 21, and those over the age of 40 years had a higher mortality rate. Several studies reported that after suffering thermal injury, females had a stronger likelihood for mortality compared to males (Karimi et al., 2017; Kerby et al., 2006; Mcgwin et al., 2002; Vico & Papillon, 1992; Benmeir et al., 1991; Tobiasen et al., 1982a). Determinants relating to the reported gender disparities were limited (Mcgwin et al., 2002; Kerby et al., 2006; O'Keefe et al., 2001), but were suggestive of differential immunological responses (Kerby et al., 2006; Gregory et al., 2000b), body fat composition (Karimi et al., 2017), hormonal variations (Jeschke et al., 2008; Kerby et al., 2006; Gregory et al., 2000a), and etiology and burn depth (Muller et al., 2001). Behavioural and development patterns have also reportedly contributed to the age-related mortality rate. Risk-taking activities with fire were

typically observed in younger individuals (García-Díaz et al., 2022) that tend to comprise the generally active and productive cohort (Shankar et al., 2010). As a result, flame burns were a frequent source of injury in these patients (García-Díaz et al., 2022; Jordan et al., 2022), which was accompanied by more severe injury, inhalation injury (Jordan et al., 2022), and complications (García-Díaz et al., 2022), factors known to increase fatality likelihood. Moreover, higher mortality rates have also been reported with elderly patients (Gill & Martin, 2015). Susceptibility was related to physical or cognitive disability with the progression of age and the pathophysiologic consequences of the injury when considering the patient's frailty (Peck, 2011; Waller et al., 1998). The physical limitations prevent these patients from evading domestic fires, and when frailty (including thinner skin) was considered (Waller et al., 1998), even smaller burns could have fatal outcomes (Alden et al., 2006). Studies have thus demonstrated the impact of gender and age on survivability. Subsequently, the former was suggested to be considered as a mortality co-factor (Grunwald & Garner, 2008; Muller et al., 2001; O'Keefe et al., 2001) and the latter be considered as an absolute (Brusselaers et al., 2010; Colohan, 2010; Smith et al., 1994) and compounding co-factor, particularly within the elderly aged (over 60 years) (Brusselaers et al., 2010; Germann et al., 1997; Smith et al., 1994; Anous & Heimbach, 1986). Moreover, the inclusion of both factors into routinely used scoring system (ABSI) at TBH and other routinely used systems for mortality prediction further highlighted the significance that these factors may have on injury outcomes.

Temporal variables revealed that increased burn injuries and mortality rates commonly occurred during the Western Cape's colder and wetter seasons (autumn and winter). This was reported as a high-risk period (Walls et al., 2017; Gevaart-Durkin et al., 2014; Blom et al., 2011; Van Niekerk et al., 2009) with incidences attributed to increased heat and energy usage in domestic settings (Van Niekerk et al., 2004). Energy poverty was experienced in SA's dense, low-income settlements (Sustainable Energy Africa, 2014); and related circumstances such as shack fires, flame-related assault, and the use of kerosene or other accelerants were the reported main etiologies of a large proportion of patients admitted to TBH (Maritz et al., 2012). Fire was also the most common source of injury in this study which could relate to its domestic use as a heat source during these colder and wetter seasons and their related domestic settings. Seasonal incidences also coincided with school vacation periods (Van Niekerk et al., 2009) and on weekdays which potentially implicated social and work-related activities, respectively. It should be noted that although stipulated as a weekday, Fridays also denoted the beginning of the weekend (Blom et al., 2016; Blom et al., 2011; Van Niekerk et al., 2009). When this was considered in the current study, the incidences were significantly higher on weekends, which was preferential for recreational or social activities. Thus, the combination of vacation and/or recreational activities, colder environments, and unsafe energy resource usage may have resulted in or exacerbated thermal events. Referral patterns indicated that the majority of patients were referred from areas within the CoCT. This was unsurprising, since TBH is located within this district. However, mortality rates were higher for patients travelling longer distances referred from regions outside the CoCT. TBH is the only provincial hospital with a dedicated specialized Burns Unit for adults and a provincial referral criteria consisting of

prognosticating factors (Sheppard et al., 2011) that contributes to triaging patient referrals for admission (Boissin et al., 2017; Karpelowsky et al., 2007). The factors were indicative of poorer clinical outcomes, and thus, the delayed treatment from prolonged referral resulted in the observed mortality rate for patients that were already critically injured. The adverse outcome may also be dependent on the availability of infrastructure and services during the clinical route (the referring and the referred clinical settings). The WCPATBC's large catchment area and the shortage of bed availability relative to the number of cases requiring treatment may have increased the waiting period for admission (Boissin et al., 2019). Moreover, a shortage of ICU beds with secondary-level care also resulted in the inability to isolate burn patients in an already unspecialized treatment setting (Rode et al., 2015). Although beyond the scope of this study, these challenges could have also played a role in mortality occurrence.

Additional pathologies from delayed admissions (Mashavave et al., 2020; Dhopte et al., 2017) have further predisposed patients to complications shown to elevate mortality (Chen et al., 2018; Lachiewicz et al., 2017; Liodaki et al., 2015; Dancey et al., 1999). Burn injuries alter important physiological functions of cutaneous tissues, causing the host's defences to become impaired and resulting in immunosuppression (Church et al., 2006; Cook, 1998). Therefore, a common complication of burn patients was the susceptibility to infections (Chen et al., 2018; Lachiewicz et al., 2017). The fatality rate was reported to increase in the presence of complications with the burns' cohort (Rech et al., 2019; Greenhalgh, 2017), an outcome that was clearly observed in this study. Contracting burn wound infections have also been previously linked to burn injury size (Lachiewicz et al., 2017; Alp et al., 2012; Church et al., 2006; Santaniello et al., 2004; Sheridan, 2000). Inclusive of the WCPATBC's admission criteria were patients with > 20% TBSA burns (Rode et al., 2014). Majority of the admitted patients had ≤ 40% TBSA burns, which were still serious considering ≥30% TBSA was the reported minimum indication for the presence of serious burn wounds (Gevaart-Durkin et al., 2014). However, increased risk of mortality was associated with patients who sustained > 40% TBSA. In similar settings, increased fatality risk has been reported in patients with burns ≥30% TBSA (Jugmohan et al., 2016; Den Hollander et al., 2014). Moreover, the presence of larger TBSA had a direct relationship to increasing mortality rates, an observation not only supported in literature by the average fatal TBSA in the higher ranges of 30-63.5% (Den Hollander et al., 2014; Maritz et al., 2012; Allorto et al., 2009; Eyal et al., 2007; Jiburum & Olaitan, 2005; Mzezewa et al., 1999; Bauling et al., 1992), but further supported with a reduced rate of mortality in patients with less severe TBSA (Allorto et al., 2009; Jiburum & Olaitan, 2005).

TBSA has strongly been associated with inhalation injury (Monteiro et al., 2017; Godwin & Wood, 1998; Tredget et al., 1990), both being major co-factors of mortality (Jones et al., 2017; Lachiewicz et al., 2017; Monteiro et al., 2017; Sheppard et al., 2011; Brusselaers et al., 2010; Santaniello et al., 2004; Smith et al., 1994). The gravity of inhalation injury relative to mortality risk was emphasized after it was suggested that with severe flame burns, the presence of inhalation injury should always be considered (Schaefer & Nunez Lopez, 2020) even prior to confirmation. The relationship between

inhalation injury, shack fires, and flame burns was previously reported (Cloake et al., 2017). Combustible materials, resultant toxic fumes and confined space associated with informal dwellings created ideal scenarios allowing fire to spread exponentially. Only a small window of opportunity would allow victims to escape, thereby increasing flame exposure. This may explain the presence of inhalation injury observed in relation to serious burns (Peck et al., 2008; Godwin et al., 1997) in the current findings. Interestingly, Gevaart-Durken et al, (2014) observed elevated TBSA (≥30%) and inhalation injury in similar age groups that dominated injury incidence, further supporting the current observations (Gevaart-Durkin et al., 2014). In addition to gender, these factors (i.e. TBSA, inhalation injury, and age) were incorporated into the routinely used ABSI scoring system which prognosticated patient survival. This study demonstrated that these variables were all related to higher mortality rates and potentially validated the ABSI's prognosticating function. The median ABSI score attained in this study demonstrated the presence of serious injuries and was higher compared to studies in similar settings (Boissin et al., 2019; Smith et al., 2016). This may have been as result of the exclusion of underlying comorbidities and factors from the calculation, which may have contributed to fatalities with less severe prognosticated scores and resultant prolonged hospital stays. Only a few relatable studies have reported on ICU LOS, and, of those, the values have been shown to vary. Averages of 3.2 (Chukamei et al., 2021) and 8.5 (Morobadi et al., 2019) days were noted, while Angelou et al, (2022) reported a 17-day median LOS (Angelou et al., 2022). Although our LOS was similar to Morobadi et al, (2019), differences were expected since the nature of the injury and the resultant outcome may vary among patients and settings. Longer LOS and its relation to adverse outcomes (Angelou et al., 2022; Boissin et al., 2019) and high mortality rates were previously noted in ICU patients (Morobadi et al., 2019; Maritz et al., 2012; Godwin & Wood, 1998) and have also been reported in this study.

As per TBH protocol, throughout LOS, and, importantly, shortly or immediately after admission, the oxygen and metabolic status of the patients were determined through measured BGA parameters. By assessing these measurements throughout the clinical course of the injury, the clinicians were able to efficiently identify, manage, and treat the related metabolic and respiratory outcomes. Elevated average PaO_2 and reduced lactate levels were observed for the total admissions, while the remaining parameters fell within normal ranges. Oxygen-related management had been critical because of the resultant low PaO_2 that required treatment by temporarily increasing FiO_2 until satisfactory levels were reached. This mitigated for oxygen anomalies and aided in any potential CO clearance (Wetterslev et al., 2015). Since the BGA measurements were performed on or shortly after patient admission at TBH, the observed hyperoxia could be in response to increased FiO_2 in the initial treatment stages when injury management was critical. Furthermore, the observed acidotic average may have influenced the oxygen binding affinity. Acidosis is reported to cause a right shift on the oxyhemoglobin curve, which is indicative of low binding affinity for hemoglobin and sporadic oxygen release. This released oxygen could have contributed to the observed PaO_2 for this given measurement. The concurrent lower lactate levels were not surprising, since as a by-product of cellular respiration, elevated lactate levels would result in

response to insufficient oxygen during glycolysis and pyruvate conversion (Suistomaa et al., 2000). Since PaO_2 was elevated, a reduced lactate level would be a potential direct consequence. The reported inverse relationship of lactate with PaO_2/FiO_2 levels was previously reported (De Backer et al., 1997). Relative to the increased mortality rate, these measurements were indicative of acidosis (low pH), severe hypoxia (very low PaO_2), hyperlactatemia (high lactate), and decreased base excess. These results demonstrated the presence of type A hyperlactatemia (Scrutton, 1976) with severe hypoxia being the likely causative factor for excess lactate production (De Lucas et al., 2020). In addition, the hyperlactatemia together with the acidotic state of patients was collectively suggestive of both lactic and metabolic acidosis as the primary condition. This deduction was also supported by the base excess results. The latter measurement provided information on the presence of all bases (mostly bicarbonate ions due to its predominance), and since these were reduced (indicating low alkaline agents), metabolic acidosis was confirmed (Burns, 2014). These findings demonstrated BGA outcomes in response to standard treatment, as well as in deceased patients or patients that were most likely to succumb to the injuries.

This chapter provided a comprehensive report on scarcely available literature on burn patients admitted to TBH's WCPATBC in Cape Town, South Africa. While the scope mainly focused on descriptive factors within South African-based clinical settings, our findings mirrored those of numerous LMICs (both locally and internationally). Sociodemographic factors that accounted for the higher admissions to TBH were also observed in burn centres from Israel (Haik et al., 2007), Turkey (Türegün et al., 1997), Cairo, Nairobi, Ibaden, Dhaka, Kathmandu, Sao Paulo, Guadalajara (Quinn et al., 2023), Nepal (Sharma et al., 2015), Eastern Sri Lanka (Laloë, 2002), and Iran (Panjeshahin et al., 2001); while the same temporal patterns were reported for admission periods to burn centres from Egypt (Mabrouk et al., 2003), Nigeria (Kalayi, 2001), Malawi (Samuel et al., 2011), Israel (Haik et al., 2007), and the Caribbean (Frans et al., 2008). This demonstrated that burns continue to remain a burden of disease, a discrepancy that has been dependent on the income of the country and the potential availability of dedicated or specialized burn treatment services. Therefore, the importance of identifying these admission patterns within LMICs would allow burn prevention strategies to be tailored from standardized HIC programmes that have effectively reduced burn incidences. Although the socioeconomic characteristics were similar, burns-associated characteristics differed between regions (Karami et al., 2012) and for a myriad of reasons (Evers et al., 2010), i.e. variations in etiology, exposure duration, presence of co-morbidities, immunological impairment, immediate specialized treatment, etc. The differences in factors indicative of injury severity and clinical outcomes continue to play a pivotal role in prognosticating survivability. Some (i.e. age, gender, and TBSA) have consistently been used in related scoring systems, while a few (i.e. LOS and lactate levels) utilized as indicators for severity progression. The presence of inhalation injury was also consistently emphasized as an important cofactor that not only elevated the mortality and morbidity rates of burn patients (American Burn Association, 2019; You et al., 2014; Lipovy et al.,

2011), but maintained these high rates due to the resultant respiratory complications (Lipovy et al., 2011).

The next chapter will focus on inhalation injury as a potential significant co-factor of mortality in the burn population at TBH's WCPATBC. However, since burn injuries have always been multifactorial in nature; all the recorded parameters, including known and suggested cofactors, were also addressed. The findings of the next chapter would not only determine the importance of inhalation injury as a contributor to mortality but also support further investigation into the condition and therefore validate the chapters that follow.

CHAPTER FOUR
INHALATION INJURY AND MORTALITY

8. Impact of inhalation injury and other burns-related variables on mortality

8.1. Overview

Mortality reported in this study was nearly 10% higher than those reaching 16% in previously reported SA-based studies (Cloake et al., 2017; Jugmohan et al., 2016; Den Hollander et al., 2014). Numerous factors were suggested to play a role (Douglas et al., 2015; You et al., 2014), with inhalation injury emphasized and proposed as one of the co-factors that largely impacted survival (You et al., 2014; Lipovy et al., 2011). Management of inhalation injury has predominantly been supportive, and varying difficulties prevented immediate diagnosis (Masanès et al., 1995), thus mortality and morbidity remained high (Lipovy et al., 2011). Literature has demonstrated the impact of inhalation burns on patient outcomes; therefore, assessing this co-factor and its relationship with mortality within the context of this study could identify additional co-factor/s correlations that may amplify the risk of mortality as well as highlight and/or verify its importance and thus support its further analysis in the chapters that follow. The main objective of this chapter was to determine the relationship between inhalation injury and mortality of the hospitalized burn population at TBH's WCPATBC (from April 2016 to August 2017). Moreover, since numerous factors affect mortality outcomes concurrently, the secondary objective included investigating relationships with the remaining sociodemographic, burn injury, and clinical variables. This could bring attention to factors that were not previously considered for the inclusion of mortality prognostication. In the study, inhalation injury was stratified into mild inhalation injury and severe inhalation injury based on the total days of patient ventilation (mild = 0-4 days; ≥ 5 days = severe), while the additional burns-related parameters recorded on admission or shortly thereafter, as reported in the previous chapter, included the following: *(i)* sociodemographic factors *viz*. gender, age, day on which the incidence occurred, the seasonal variation of the incidence, the district the patient was referred from, and the clinical level of referral; *(ii)* injury characteristics *viz*. aetiology, %TBSA, phenotypical characteristics, burns-related complications, and ABSI scores; and *(iii)* clinical parameters *viz*. BICU LOS, ventilation before admission, and ABG measurements (pH, PaO_2, PCO_2, Sats, lactate, and BE levels). The relationship between mortality and the above-mentioned parameters was assessed in the following way: in order to determine significant association, the Fisher's Exact test was utilized and the Pearson Point-Biserial and/or Spearman's Rank correlation coefficient tests were used to determine the relationship strength and direction of correlations. Partial least squares regression was then used to model the significant correlations and determine the best mortality predictors based on specific output values.

8.2. Results

8.2.1. Relationship between burns-related variables and mortality

Inhalation injury was significantly associated (P=0.005) and correlated (P<0.000) with mortality. The rho value (0.441) was indicative of a positive and strong correlation. Marked associations were also observed for referral setting/district (P=0.038), %TBSA (P<0.001), complications (P<0.001), ABSI scores (P<0.001) and BICU LOS (P=0.007). These factors also significantly correlated with mortality, in addition to hoarseness (P=0.038; CI=-0.499-0.009). The size/strength and direction of correlations determined that all the factors positively correlated with mortality, barring hoarseness. Furthermore, strong correlations were also observed with complications (rho=0.681), ABSI scores (rho=0.558), and %TBSA (r_{pb} =0.554). A moderate correlation was observed with BICU LOS (rho=0.326), while weak correlations for referral settings/district (rho=0.294) and hoarseness (rho=-0.271) were noted (**Table 1**).

Table 1: Association and correlation between the burn-related variables and mortality

Study variables	Fisher's *p*-value	Correlation coefficient	r_{pb}/rho *p*-value	95% CI
Socio-demographic factors				
Gender (male/female)	0.759	rho = 0.054	0.686	-0.213-0.313
Age (years)	0.082	r_{pb} = 0.081	0.543	-0.179-0.330
Day of the week (week/weekend days)	0.537	rho = -0.113	0.393	-0.366-0.155
Seasonal variation (colder/warmer)	0.762	rho = 0.068	0.611	-0.199-0.325
Referral setting (COCT/other districts)	0.038*	rho = 0.294	0.024*	0.033-0.517
Referral Level (hospitals or CHC/clinics)	0.721	rho = 0.065	0.623	-0.202-0.323
Days between injury & admission (days)	0.092	rho = -0.027	0.837	-0.289-0.238
Injury characteristics				
Aetiology (fire/other sources)	0.085	rho = 0.252	0.055	-0.013-0.483
TBSA (%)	<0.001*	r_{pb} = 0.554	<0.000**	0.347-0.709
Inhalation injury (mild/severe)	0.005*	rho = 0.441	<0.000**	0.200-0.631
Singed nasal hairs (yes/no)	0.753	rho = -0.075	0.571	-0.332-0.192
Soot around/in mouth (yes/no)	0.207	rho = -0.181	0.171	-0.424-0.087
Hoarseness (yes/no)	0.059	rho = -0.271	0.038*	-0.499-0.009
Facial burns (yes/no)	0.666	rho = 0.094	0.480	-0.174-0.349
Complications (yes/no)	<0.001*	rho = 0.681	<0.000**	0.509-0.801
ABSI scores (4-13 points range)	<0.001*	rho = 0.558	<0.000**	0.346-0.716
Clinical parameters				
BICU LOS (days)	0.007*	rho = 0.326	0.012*	0.069-0.543
Ventilation prior to admission (yes/no)	0.666	rho = 0.118	0.375	-0.150-0.369
pH	0.189	r_{pb} = -0.206	0.117	-0.439-0.053
PaO_2 (kPa)	0.537	r_{pb} = 0.111	0.404	-0.150-0.357
PCO_2 (kPa)	0.858	r_{pb} = -0.114	0.389	-0.360-0.146
Sats (%)	1.000	rho = 0.053	0.691	-0.213-0.312
Lactate (mmol/L)	0.137	rho = 0.232	0.077	0.033-0.467
Base excess (mmol/L)	0.579	rho = -0.032	0.810	-0.293-0.233

R_{pb} – Pearson's point biserial coefficient, rho – Spearman Rank's coefficient, CI – confidence interval, CoCT – City of Cape Town, CHC – community health centre, TBSA – total body surface area, ABSI – abbreviated

burn severity index, BICU LOS – burns intensive care unit length of stay, PaO_2 – oxygen partial pressure PCO_2 – carbon dioxide partial pressure.
Pearson's r_{pb} interpretation: ±0.1 (small/weak); ±0.3 (medium), ±0.5 (large).
Spearman's rho interpretation: 0.01 to 0.19 (no or negligible); 0.20 to 0.29 (weak), 0.30 to 0.39 (moderate); 0.40 to 0.69 (strong); ≥0.70 (very strong).
Significance: *: Correlation is significant at the 0.05 level (2-tailed); **: Correlation is significant at the 0.01 level (2-tailed).

8.2.2. Burns-related variables as predictors of mortality

The initial regression model for predicting mortality incorporated the presence of significantly correlated factors (**Table 2**), while the secondary model consisted of the sub-groups of those factors, when applicable (**Table 3**). Latent factor 1 for both models demonstrated the best described variances with the highest R^2 values. These respective VIP values were annotated for each regression model. The results demonstrated that inhalation injury was associated with a significant VIP value (0.8) along with referral setting and BICU LOS. The highest values were attributed to complications (VIP=1.4), ABSI scores (VIP=1.2) and %TBSA (VIP=1.1) (**Table 2**).

Table 2: Predicted variances and VIP values generated by PLS regression incorporating significantly associated and correlated independent burn-related variables for the mortality prediction model

	Predicted variances of latent factors				
	1	**2**	**3**	**4**	**5**
***X*-variance**	0.517	0.201	0.102	0.092	0.065
***Y*-variance**	0.475	0.064	0.048	0.009	0.007
Adjusted R-square (R^2)	0.465	0.465	0.564	0.566	0.566
	Latent factors VIP values per variable				
Referral setting	0.603	0.573	0.557	0.570	0.572
Complications	1.398	1.385	1.480	1.469	1.463
%TBSA	1.138	1.070	1.074	1.069	1.062
Inhalation injury	0.819	0.807	0.797	0.820	0.870
ABSI scores	1.205	1.142	1.108	1.099	1.096
BICU LOS	0.515	0.824	0.884	0.895	0.924

VIP – variable importance in prediction, PLS – partial least squares, TBSA – total body surface area, ABSI – abbreviated burn severity index, BICU LOS – burns intensive care unit length of stay.

Prior to the analysis of the sub-groups, ABSI sub-groups were assessed separately in order to determine which score contributed significantly to mortality. The less severe ABSI scores had VIP values >0.5, while severe and maximum ABSI scores had higher VIP values of 1.5 and 1.3, respectively (**Appendix A: Table 12**). Only the higher-scored ABSI sub-groups were re-introduced into the secondary model for the following reasons: (*i*) it eliminated potential noise that influenced statistical output, (*ii*) it was associated with increased mortality likelihood in literature, and (*iii*) there was a strong correlation with increasing ABSI scores in this study. The secondary model demonstrated significant VIP values for severe inhalation injury (0.8) along with both ABSI scores, BICU LOS ≥ 10 days and referral settings outside of CoCT. ABSI sub-groups were also the only variables that did not retain similar VIP values compared to its inclusion in the initial model as a continuous variable, but still remained high. However,

the presence of complications (VIP=1.4) and large %TBSA (VIP=1.3) burns had the highest values (**Table 3**).

Table 3: Predicted variances and VIP values generated by PLS regression incorporating the sub-groups of the significantly associated and correlated independent burn-related variables for the mortality prediction model

	Predicted variances of latent factors				
	1	**2**	**3**	**4**	**5**
***X*-variance**	0.392	0.148	0.141	0.117	0.087
***Y*-variance**	0.591	0.045	0.012	0.005	0.001
Adjusted R-square (R^2)	0.584	0.623	0.629	0.627	0.622
	Latent factors VIP values per variable				
Referral setting (other districts)	0.617	0.599	0.595	0.598	0.601
TBSA group (≥40%)	1.321	1.301	1.290	1.285	1.285
Inhalation injury degree (severe)	0.813	0.899	0.898	0.895	0.895
Complications (present)	1.431	1.392	1.405	1.410	1.409
ABSI severe (10-11) score	0.948	0.950	0.943	0.953	0.953
ABSI maximum (12-13) score	0.833	0.803	0.834	0.833	0.833
BICU LOS (≥10 days)	0.757	0.817	0.811	0.816	0.817

VIP – variable importance in prediction, PLS – partial least squares, TBSA – total body surface area, ABSI – abbreviated burn severity index, BICU LOS – burns intensive care unit length of stay.

8.3. Discussion

Findings revealed that the burns-related factors that were significantly associated with, correlated to and/or predicted for mortality were both known and suspected co-factors of mortality. Moreover, the unexpected "cross-talk" between the statistically employed tests was confirmed with the significant predictors reflecting the strong correlation and association outcomes. Six variables were identified as potential clinical predictors for mortality, with one currently used as a tool for prognostication, which was also a potential expected outcome. Inhalation injury was one of two of the known cofactors of mortality whose presence in burn patients has been emphasized in both literature and in the current study. Mild inhalation injury was associated with only 1 of the 15 deceased patients; therefore, not only was severe inhalation injury present in nearly the entire mortality population, but the results also demonstrated the significant relationship and predictive capacity for mortality. These results were pivotal since it both aligned with previous findings and provided affirmation for further investigation in the current study population. Although not typically related to mortality, the presence of the included phenotypical characteristics (facial burns, hoarseness, etc.) would increase clinical suspicion of inhalation injury presence and therefore indirectly affect mortality risk through the impact of inhalation injury. Hoarseness had a significantly negative and weak correlation; however, this was attributed to the statistical phenomenon referred to as 'spurious or nonsense correlation' (Atkinson et al., 2004; Pearson, 1897). For further context, these correlation types have been recognized as statistical trends without a pathological basis. Since no clinical inferences could be made by the absence of hoarseness contributing to mortality, a spurious correlation was concluded. Moreover, hoarseness was the only variable lacking an association with the Fisher's test, which could provide some statistical support for the observed weak correlation and resultant spurious outcome. The other known cofactor, TBSA (Jones et al., 2017;

Lachiewicz et al., 2017; Monteiro et al., 2017), was one of two of the strongest predictors in this study. Representing the cutaneous and physical aspect of injury that would immediately provide an initial prognosis for injury degree, extensive TBSA burns would mirror not only severity, but also the patient's predisposition to mortality. Furthermore, the LA50 was also assessed, which demonstrated a percentage relative to TBSA expected to result in 50% of burns-related deaths (Bull & Squire, 1949). This index served as a reliable tool used to measure the degree of disease and hospital care quality (Seyed-Forootan et al., 2016; Jie et al., 2003). Higher values, suggestive of improved burns management, were attributed to HICs (Bull & Squire, 1949) having reached a LA50 value (Kasten et al., 2011) more than double compared to LMICs (Kiragu et al, 2018; Tyson et al 2013). These differences have been attributed to numerous factors (Mathonsi & Arko-Cobbah, 2023) that created challenges to the detriment of burns management and care in LMICs. The LA50 of 37.4% was observed with the admitted burns population in this study, which aligned with those reported at other South African-based burn centres (Mathonsi & Arko-Cobbah, 2023; Boissin et al., 2019; Allorto et al., 2018). These consistently low LA50 values may demonstrate the need for improving or stringently implementing burn strategies and preventative training programs. Moreover, highlighting the necessity of dedicated financial assistance for developing and employing uniform quality services in public hospitals where adult burn patients would be treated in non-dedicated or specialized burn centres.

Four additional variables were significant for risk of mortality: ABSI scores, complications, BICU LOS, and referral district. At TBH, ABSI has been implemented as a standard survival prognostic tool, which in turn also incorporated two identified co-factors: inhalation injury and TBSA. The strong relation to mortality was therefore not only unsurprising, but its prognostic use was validated. Complications had the strongest correlation and predictive value for mortality. Since complications were present in the entire mortality population even crude analysis demonstrated its significance. The fatality rate reportedly increased in the presence of complications with the burns' cohort (Rech et al., 2019; Greenhalgh, 2017), which could explain why all the deceased patients had some form of associated complication. Septic shock was present in all 15 mortality cases with the concurrent presence of ARDS in 10 cases. Sepsis-related deaths in adult burn patients were reported to be between 49.4 and 84% (Rech et al., 2019; Nunez Lopez et al., 2017; Gomez et al., 2009; Dasari et al., 2008; Sharma et al., 2006) which supported the high frequency observed in the current study and further demonstrated the ability of these burn-associated infections to increase the likelihood of death in an already vulnerable burn population (Dhopte et al., 2017; Krishnan et al., 2013; Keen III et al., 2010). Despite its strong contribution, this parameter has not typically been considered in prognostic scoring systems, including for ABSI, most likely due to varying onset and development during the LOS. However, it would be a valuable indicator in patients that were referred from areas distal to the treating hospital. An ideal and opportunistic window may result from delayed admissions, especially for patients with severe injuries and poor prognosis that require referral and specialized critical care. Previous reports have observed unfavorable outcomes with admission delays (Mashavave et al., 2020; Dhopte et al., 2017) and also reported a

predisposition to burns-associated complications that elevated mortality risk (Chen et al., 2018; Lachiewicz et al., 2017; Liodaki et al., 2015). It can be suggested that in the prolonged absence of specialized burn care, critical patients would have a higher mortality likelihood compared to those receiving more immediate treatment or shortly after incidence due to proximity. Albeit it a weak relationship, significant mortality correlation and prediction were observed for the patients referred from areas outside of the CoCT (TBH's district). The same was observed for BICU LOS, which has been affiliated with higher mortality rates in similar settings (Morobadi et al., 2019; Maritz et al., 2012; Godwin & Wood, 1998). Moreover, prolonged LOS has typically been a consequence of severe injury in burn patients and has been considered a notable outcome measure (Onah et al., 2021) and predictor for mortality (Jafaryparvar et al., 2021). The impact of extended hospital stay has gained attention, with more studies focusing on finding predictors or contributors for the parameter itself (Chukamei et al., 2021; Onah et al., 2021; Dolp et al., 2018; Taylor et al., 2015). It is plausible that LOS would not only reflect adverse clinical outcomes; but also indicate transport, social, and economic-related issues (Boissin et al., 2019).

Interestingly, the presence of one of the above-analyzed factors was nearly always connected to another. It was noted that complications were known consequences of inhalation injury (Shubert & Sharma, 2018), TBSA (Ali & Ali, 2022), LOS (Chen et al., 2018), and referral delay (Chen et al., 2018); while LOS increased when burns were more severe and inhalation injury was present (Dolp et al., 2018). This study demonstrated that, when these parameters were observed in patients who died after distal referral to the centre; complications, longer BICU LOS, ABSI scores ≥ 8 (serious and above), larger burns (TBSA ≥27%), and severe inhalation injury were always presented. For the referred patients that survived, the only parameter consistently absent was complications. This demonstrated that burn injuries were far too multifactorial and woven for a single clinical marker to be definitively assigned for mortality prognostication. However, the presence of more than one of these parameters may be reliable performance indicators based on the direct relationships with injury progression and poor survival outcomes. The risk of burns-related mortality may therefore be reduced by minimizing the key factors identified in this study. The primary outcome, inhalation injury, demonstrated statistical relevance, with literature having further demonstrated reduced mortality rates in its absence. These findings were used as the basis and rationale for further investigation into identifying potential clinical markers for inhalation injury presence or degree utilizing the same statistical testing.

CHAPTER FIVE
BURNS-RELATED VARIABLES AND INHALATION INJURY

9. Burns-related variables for the prognostication of inhalation injury presence

9.1. Overview

Inhalation injury observed in this study strongly correlated and significantly predicted burns-related mortality. Public hospitals, such as TBH, have typically been challenged with the availability of equipment or machinery required to generate the grading systems used to determine inhalation injury presence and severity, i.e. the AIS (Endorf & Gamelli, 2007) and RADS (Oh et al., 2012; Park et al., 2003). Moreover, due to inhalation injury's multifactorial nature, outcomes have been dependent on specific factors (Sousse et al., 2015; Walker et al., 2015) not typically recorded in the physical and history evaluation during injury assessment, creating additional prognostic challenges. Clinicians primarily focus on identifying cofactors recorded in the patient's physical and history evaluation as well as their clinical measurements to determine survivability. However, if these measurements could also prognosticate for the known co-factors of mortality, such as inhalation injury, then survivability could indirectly be improved as well. Some of these factors were proposed as potential predictors for inhalation injury-associated complications (Mokline et al., 2017; Herrero et al., 2015), which also supported a need for further investigation. Identifying potential clinical factors with strong prognostic function for inhalation injury would be highly beneficial in a clinical setting. The advantage of accessing this data lies in it already having been recorded as part of the standard routine executed shortly after the patient's admission. Thus, the objective of this chapter was to describe the hospitalized burn population at TBH's WCPATBC with mild and severe inhalation as well as to identify potential clinical markers for inhalation injury by assessing relationships using the burns-related variables and statistical methods previously described in Chapter 4. Inhalation injury was stratified into patients with mild and severe inhalation injury based on the total days of ventilation (mild = 0-4 days; ≥ 5 days = severe). The burns-related parameters analyzed included: *(i)* sociodemographic factors *viz*. gender, age, day on which the incidence occurred, the seasonal variation of the incidence, the district the patient was referred from, and the clinical level of referral; *(ii)* injury characteristics *viz*. aetiology, %TBSA, phenotypical characteristics, burns-related complications, and ABSI scores; and *(iii)* clinical parameters *viz*. BICU LOS, ventilation before admission, and ABG measurements (pH, PaO_2, PCO_2, Sats, lactate, and BE levels). The following statistical tests were used to determine the significant relationships between inhalation injury and the parameters of interest: *(i)* the Fisher's Exact test for significant association, *(ii)* the Spearman's Rank coefficient test for correlation strength and direction, and *(iii)* PLS regression for predictors of inhalation injury presence and/or degree (see Chapter 2: Methodology).

9.2. Results

9.2.1. Sociodemographic, injury and clinical factors of burns patients with mild and severe inhalation

Patients with severe inhalation injury represented 61% of the total admissions with an average of 11.2 patients (CI=9.5-12.9) and a 2.6-fold predominance in favour of males. The 21-39-year age group was most affected by both degrees of inhalation injury, with near identical mean ages for both mild (mean=32.6; CI=27.5-37.6) and severe inhalation injury (mean=32.9; CI=29.5-36.5). Both degrees were also predominantly sustained on weekdays, during the colder seasons, flame etiology and with patients referred from hospital settings and within the CoCT district. Smaller burns areas (≤40% TBSA) were observed for all the mild (mean=16.3; CI=11.5-21.2) and most of the severe cases (mean=27.8; CI=22.5-33.1). Regarding the four phenotypical characteristics, patients with mild inhalation injury (median=3.0; IQR=2.0-4.0) had the same median value as the severe cases (median=3.0; IQR=2.0-3.0). Facial burns were most frequently observed (mild: 82.6%; severe: 91.7%) and hoarseness the least (mild: 52.2%; severe: 25.0%). Severe inhalation injury-related patients presented with the most complications (>63%). Lower ABSI scores (≤7) were associated with majority of the mild group (61%), while most of the severe population (67%) had ABSI scores ranging from serious to maximum (median=8.3; IQR: 7.6-8.9) (**Appendix B, Tables 13 and 14**).

Both injury groups had a high and similar occurrence of ventilation prior to admission to the WCPATBC (mild: 87%; severe: 86.1%). Patients with mild inhalation injury demonstrated strong association (87.0%) with a ≤9-days BICU LOS (median=5.0; IQR=3.0-6.0) compared to the severely affected patients who spent a min of 10 days (mean=12.0; IQR=9.0-11.0) in the BICU. With the exception of elevated PaO_2 levels (56.5%; mean=16.8 kPa; CI=14.1-19.6), majority of the patients with mild inhalation injury had normal ranges for the remaining ABG parameters, these included: pH (52.2%; mean=7.4; CI=7.3-7.4), PCO_2 (47.8%; mean=5.3; CI=4.8-5.9), Sats (87.0%; median=98.0; IQR=96.0-99.0), lactate (73.9%, median=1.4 mmol/L; IQR=0.7-2.4) and base excess (65.2%; mean=-2.3; CI=-3.8--0.7). With the severe inhalation injury cases, the following blood gas parameters were within the normal ranges: PCO_2 (50.0%; mean=5.5 kPa; CI=5.1-5.9), Sats (88.9%; median=99.0%; CI=98.0-100.0) and base excess (72.2%; median=-1.7 mmol/L; IQR=-3.7-0.1). Elevated PaO_2 and lactate levels were also observed (77.8%; mean=19.9 kPa; CI=17.0-22.6 and 52.8%; median=2.0 mmol/L; IQR=1.3-2.6, respectively) (**Appendix B, Table 15**).

9.2.2. Burns-related clinical variables and inhalation injury

Significant associations between inhalation injury and the following factors were observed: % TBSA (P=0.008), complications (P<0.001) and BICU LOS (P<0.001). These variables also demonstrated significant correlation to ABSI scores, lactate levels, and hoarseness. All the parameters displayed positive relationships, with the exception of hoarseness (which showed a negative correlation). BICU

LOS and complications had a very strong (rho=0.908; P<0.001; CI=0.847-0.945) correlation and strong correlation (rho=0.690; P<0.001, CI=0.522-0.807) with inhalation injury, respectively. The remaining variables were moderately correlated as follows and in descending order: %TBSA (rho=0.357; P=0.006; CI=0.103-0.567), ABSI scores (rho=0.347; P=0.007; CI=0.093-0.560), lactate levels (rho=0.331; P=0.011; CI=0.074-0.556) and hoarseness (rho=-0.314; P=0.015; CI=0.093-0.560) (**Table 4**).

Table 4: Association and correlation between the burn-related variables and inhalation injury

Study variables	Fisher's *p*-value	Correlation Coefficient (rho)	rho *p*-value	95% CI
Socio-demographic factors				
Gender (male/female)	0.350	-0.049	0.712	-0.308-0.217
Age (years)	0.080	0.095	0.474	-0.173-0.350
Day of the week (week/weekend days)	0.379	-0.006	0.963	-0.269-0.258
Seasonal variation (colder/warmer)	0.421	-0.013	0.920	-0.276-0.251
Referral setting (COCT/other districts)	0.161	0.151	0.252	-0.117-0.399
Referral Level (hospitals or CHC/clinics)	0.082	0.107	0.419	-0.161-0.360
Days between injury & admission (days)	0.311	-0.109	0.409	-0.289-0.238
Injury characteristics				
Aetiology (fire/other sources)	0.351	0.118	0.373	-0.150-0.370
TBSA (%)	0.008*	0.357	0.006**	0.103-0.567
Singed nasal hairs (yes/no)	0.472	0.068	0.611	-0.199-0.325
Soot around/in mouth (yes/no)	0.908	0.046	0.730	-0.220-0.306
Hoarseness (yes/no)	0.178	-0.314	0.015*	-0.533-0.055
Facial burns (yes/no)	0.750	0.091	0.492	-0.176-0.346
Complications (yes/no)	<0.001*	0.690	<0.000**	0.522-0.807
ABSI scores (4-13 points range)	0.163	0.347	0.007**	0.093-0.560
Clinical parameters				
BICU LOS (days)	<0.001*	0.908	<0.001**	0.847-0.945
Ventilation prior to admission (yes/no)	0.267	0.019	0.887	-0.246-0.281
pH	0.936	-0.223	0.090	-0.399-0.116
PaO_2 (kPa)	0.381	0.006	0.964	-0.258-0.269
PCO_2 (kPa)	0.955	0.089	0.503	-0.179-0.344
Sats (%)	0.626	0.200	0.129	-0.067-0.440
Lactate (mmol/L)	0.060	0.331	0.011*	0.074-0.556
Base excess (mmol/L)	0.936	-0.040	0.763	-0.300-0.226

Rho – Spearman Rank's coefficient, CI – confidence interval, CoCT – City of Cape Town, CHC – community health centre, TBSA – total body surface area, ABSI – abbreviated burn severity index, BICU LOS – burns intensive care unit length of stay, PaO_2 – oxygen partial pressure PCO_2 – carbon dioxide partial pressure.
rho interpretation: 0.01 to 0.19 (no or negligible); 0.20 to 0.29 (weak), 0.30 to 0.39 (moderate); 0.40 to 0.69 (strong); ≥0.70 (very strong).
*: Correlation is significant at the 0.05 level (2-tailed).
**: Correlation is significant at the 0.01 level (2-tailed).

9.2.3. Burns-related clinical variables as predictors for inhalation injury

The significantly associated and correlated variables were assessed for their predictive contribution to inhalation injury presence and degree. As reported in the previous chapter (see Chapter 4: Inhalation injury and mortality), hoarseness was excluded from the regression model due to its spurious correlation.

ABSI scores were also omitted from the model to reduce redundancy, since inhalation injury is included as a parameter in the scoring calculation and would therefore potentially result in biased outcomes. Three regression models analyzed the independent correlated variables, inhalation injury and sub-groups of the latter and former. The first model assessed the ungrouped variables for the prediction of inhalation injury presence (**Table 5**). The second and third model incorporated the sub-groups of these variables as potential predictors for injury presence and severe degrees of inhalation injury, respectively, and the results were displayed in a combined table (**Table 6**). Severe inhalation injury degree was selected as an additional dependent condition because of its identified close association with more adverse outcomes and mortality.

Table 5: Predicted variances and VIP values generated by PLS regression incorporating significantly associated and correlated independent variables for the inhalation injury presence prediction model

	Predicted variances of latent factors for inhalation injury presence			
	1	**2**	**3**	**4**
***X*-variance**	0.509	0.216	0.087	0.188
***Y*-variance**	0.729	0.082	0.008	0.000
Adjusted R-square (R^2)	0.725	0.805	0.809	0.806
	VIP values per variable for inhalation injury presence prediction			
Complications	1.117	1.064	1.070	1.070
%TBSA	0.487	0.606	0.609	0.609
BICU LOS	1.495	1.491	1.487	1.487
Lactate	0.527	0.544	0.542	0.543

VIP – variable importance in prediction, PLS – partial least squares, TBSA – total body surface area, ABSI – abbreviated burn severity index, BICU LOS – burns intensive care unit length of stay.

For all 3 models, the VIP scores of latent factors 1 were considered based on the most variation in the Y-variables as described by the X-variables, along with the highest R^2 values. The strongest predictors for inhalation injury presence (R^2=72.5%) were BICU LOS (VIP=1.495) and complications (VIP=1.117), while VIP scores of >0.5 was observed for lactate and %TBSA (**Table 5**). The regression models incorporating the sub-grouped variables for the prediction of inhalation injury presence (R^2=59.7%) showed that BICU LOS ≥10 days (VIP=1.372) and the presence of complications (VIP=1.229) were the optimal predictors. It also indicated that TBSA >40% (VIP=0.590) and excess lactate levels (VIP=0.509) were significant contributors (**Table 6)**. These predictive outcomes were similar to those observed for the severe degree (R^2=39%) of inhalation injury. The presence of BICU LOS ≥10 days and complications continued to have the highest VIP values (1.303 and 1.229, respectively). TBSA >40% (VIP=0.662) and excess lactate levels (VIP=0.594) remained significant contributors as well (**Table 6**).

Table 6: Predicted variances and VIP values with the highest adjusted R^2 generated by PLS regression incorporating sub-groups of the significantly associated and correlated independent variables for the inhalation injury presence and severe degree prediction models

Predicted variances of latent factor 1 for inhalation injury presence and severe degree

	Inhalation injury presence	Severe inhalation injury
***X*-variance**	0.490	0.492
***Y*-variance**	0.604	0.401
Adjusted R-square (R^2)	0.597	0.390
VIP values per variable sub-groups for injury presence and severe degree prediction		
Complications (present)	1.229	1.229
%TBSA (>40 days)	0.590	0.662
BICU LOS (≥10 days)	1.372	1.303
Lactate (Excess)	0.509	0.594

VIP – variable importance in prediction, PLS – partial least squares, TBSA – total body surface area, ABSI – abbreviated burn severity index, BICU LOS – burns intensive care unit length of stay.

9.3. Discussion

Inhalation injury markedly impacted burn outcomes, which was observed by the statistically significant relationship with mortality in this study. Since it has previously been reported as a co-factor (American Burn Association, 2019; You et al., 2014; Lipovy et al., 2011), it was plausible that the likelihood of mortality would be reduced if inhalation injury was effectively identified and treated. However, diagnosis and prognosis of inhalation injury lacks uniform consensus when it comes to the application of diagnostic and scoring tools (Foncerrada et al., 2018), in part due to latent symptomology, but also because of early detection limitations with the available diagnostic modalities. Although resource-constrained clinical settings (such as the Burns Unit at TBH) provide quality care for burn patients by employing innovative strategies (combining resourceful strategies and protocols), optimizing available resources, and adhering to core principles of burn management (Tremblay et al., 2024; Cancio et al., 2017; Atiyeh et al., 2009); numerous challenges involving access and availability of diagnostic tools remain a significant barrier. The importance of this section of the study lies in the benefit for clinicians who have access to the patients' medical files to potentially make calculated and objective decisions in the hospital setting based on statistically-associated clinical data (that could be less time-dependent if an easily accessible, electronic frame-work template exists) prior to extending analysis into biomedical aspects (that would require additional tissue collection and processing). Within this clinical cohort, the results showed that it was indeed possible to determine if the patient- and injury-related recorded medical information could prognosticate for the presence or progress of inhalation injury.

On admission, data from patient history and classic symptomology were recorded as part of the clinical index of high suspicion and noted for visible indications of inhalation injury presence. In this study, inhalation injury did not significantly associate or correlate with most of the classic symptoms (singed nasal hair, soot around/in mouth, and facial burns) provided in the patient medical files. Onishi et al, (2017) also retrospectively studied patient data from medical files and found no significant correlation with singed nasal hair and hoarseness but observed associations with facial and neck burns. Only hoarseness demonstrated a negatively moderate correlation with inhalation injury in this study, which, as with the findings in the previous chapter (that focused on burns-related variables and mortality), was deduced as a spurious correlation. Interestingly, in its rational form, the pathological inference would

denote that the absence of hoarseness was related to the increasing degree or presence of inhalation injury; however, the inverse was typically considered for prognosis in a clinical setting. The negative correlation may have been as a result of the small data set not accurately representing the broader population (Bland & Altman, 1994); but in general, the variability in symptom presentation and injury incidence may be the primary reason for the inability to definitively use any singular classic symptom for inhalation injury prognostication, especially for the more severe injury form. The presence of hoarseness may indicate upper airway involvement, but damage to the mucosal region of the oropharynx is primarily caused by heat transfer, which is rarely observed since high blood flow in the upper airways would allow for rapid cooling (Jeschke et al., 2020; Rong et al., 2011; Cox et al., 2009; Baile et al., 1985). On the other hand, factors such as dehydration or vocal strain from shouting during the thermal event could also subsequently affect the upper airway mucous membranes (Sivasankar & Leydon, 2010) and result in damage to the vocal cord-containing laryngeal area (Fang-Gang et al., 2015). Thus, hoarseness can occur both dependently or independently of heat or smoke damage and could still be evident in the absence of significant airway damage. Importantly, without the presence of hoarseness, severe inhalation injury could still occur by the chemical toxic gases bypassing the larynx and causing lower airway damage. These physical signs may indeed indicate that the patient was exposed to smoke, but cannot necessarily be correlated to the severity of injury. Therefore, the initial clinical presentation data attained in this study may not accurately prognosticate the true extent of burn injuries. This is supported by Huang and colleagues, (2022) reporting that physical findings (such as singed nasal hairs, hoarseness, etc.) were not reliable indicators for facial burns patients with inhalation injury after observing the lack of predictability for the condition (Huang et al., 2022b).

Although these parameters may not conclusively prognosticate inhalation injury, their presence and progression have been critical in determining whether patients should be intubated or not. The need for early intubation had been based on reduced airway patency, which was an inhalation injury-related consequence of devolving anatomical changes in the respiratory tract and unrecognized edema (Concannon et al., 2023; Orozco-Peláez, 2018). However, endotracheal intubation (ETI) also carried several risks including damage to upper airway structures (Eastman et al., 2010), while subsequent mechanical ventilation has been linked to complications such as tracheal stenosis, ventilator-associated pneumonia (VAP), and ARDS (Moshrefi et al., 2019). Despite the need for more precise intubation criteria, potentially unnecessary ETI has been a common practice. Huang et al, (2022b) demonstrated that of the total number of patients that were intubated based on physical examination findings, only 60.3% had confirmed inhalation injury after diagnosis with bronchoscopy. Thus, the increased risk of introducing associated complications that exacerbate the patient's prognosis and delay recovery may be an outcome for a large population that did not require ETI to begin with. In this case, Huang et al, (2022b) concluded that apart from physical findings being unreliable markers for inhalation injury prognostication, they were not always indicative of the requirement for ETI (Huang et al., 2022b). The authors did, however, report that independent risk factors for inhalation injury were shortness of breath

and large TBSA burns (Huang et al., 2022b). Although shortness of breath was not a parameter included in the patient's medical history and therefore not included in this study, increasing TBSA burns significantly correlated with and predicted inhalation injury, along with longer BICU LOS, the presence of complications, and elevated lactate levels.

Prolonged BICU LOS and the presence of complications had the strongest predictive potential for inhalation injury according to regression analysis. Several studies have reported relationships with these factors and inhalation injury. Monteiro et al, (2017) reported TBSA as an independent predictive factor for lower respiratory tract injury (severe degree). TBSA may therefore be useful when inhalation injury is suspected and when considered with specific factors such as etiology and co-morbidities that may affect mobility (including younger and elderly aged cohorts). The mobility parameter may be significant in the case of flame burns, because the inability to escape due to immobility prolongs exposure to the physical (fire) and chemical (smoke) event, which could result in the concomitant occurrence of larger burns and more severe inhalation injury. Factors, such as age, should therefore be considered as secondary or indirect indicators of mobility and perhaps be considered in the statistical testing for inhalation injury presence. While the data may reflect that larger burns would almost always be accompanied by inhalation injury, its absence cannot be ruled out as patients can still be exposed to the smoke inhalants without sustaining flame burns. Since these factors could be mutually exclusive, TBSA would therefore not be a reliable independent indicator (on its own) for inhalation injury. This could explain why TBSA was a significant predictive factor, but not the strongest for inhalation injury in this study.

In addition to TBSA, Monteiro and colleagues (2017) also reported that complications, such as ARDS and pneumonia, were independent indicators in deceased patients that had inhalation injury. They further stated that ARDS had the largest impact on the prognosis of these patients. Results from this study supported the latter observation by the strong positive correlation between inhalation injury degree and complications presence, which was one of the two strongest predictors for the condition (Monteiro et al., 2017). Only two patients with mild injury had complications (both septic shock), and of the 36 patients with severe inhalation injury, 23 had complications. VAP was observed in 7 patients, which was second to the more prevalent complication (i.e. concurrent ARDS and septic shock in 9 patients). ARDS occurred in a noticeable amount of patients with thermal injury (Jones et al., 2013); however, patients with inhalation injury were more predisposed (Afshar et al., 2019). Together, ARDS and pneumonia were described as primary causative agents for respiratory dysfunction observed with inhalation injury (Bittner & Sheridan, 2023; Silva et al., 2016). Moreover, inhalation injury-induced ARDS resulted in a more adverse oxygenation-related state when compared to ARDS caused by cutaneous burns only (Lam & Hung, 2019). Concurrent septic shock was another leading complication in the current cohort of patients admitted to TBH. As the last and more serious phase of sepsis, septic shock has been associated with conditions involving perfusion anomalies despite appropriate treatment

(Hotchkiss et al., 2016). Studies have demonstrated a higher risk of sepsis occurring in patients with inhalation injury due to the resultant ALI (Rue III et al., 1995; Thompson et al., 1986). As for the second most prevalent complication identified, VAP, it had previously been noted as a frequent complication observed in lower respiratory injury (Monteiro et al., 2017). The marked incidence of pneumonia demonstrated two facets: typical distal region localization and the potential as an indicator for injury severity. It was not uncommon for burn patients to present with numerous complications (simultaneously) due to the pneumonia's pathological and multifactorial complexities. However, whether a single complication promoted the development of another would require additional analysis in order to definitively conclude a compounding and potentially more adverse impact as an indirect consequence of inhalation injury. The development of complications has been dependent on progressive immunological or injury outcomes (Le et al., 2020; Trillo-Alvarez et al., 2011) and would therefore not present immediately after incidence or even shortly after admission. Complications therefore cannot be used as an indicator for prognostication unless present on admission, which is a possibility most related to patients with delayed referral and without confirmed complications or in patients that develop complications during the referral route. The presence of any of these complications may not only allude to the presence of inhalation injury but could also indicate injury degree and be highly useful in cases where injuries were underestimated or even misdiagnosed. Since the time elapsed during referral may only be considered in the assessment and management of inhalation injuries, the findings in this study may highlight its potential inclusion, as an indirect factor for disease progress or severity onset, into a formal diagnostic or prognostic tool for inhalation injury.

Complications were also frequent in patients treated for oxygen-related abnormalities (Thompson et al., 1986) with mechanical ventilation (Moshrefi et al., 2019). Such anomalies were not only related to the perfusion state of the patient, but have also included conditions indicative of metabolic-related outcomes, such as lactic acidosis (Hotchkiss et al., 2016). More than half (approximately 53%) of the 36 patients admitted to TBH with severe inhalation injury presented with hyperlactatemia (lactate levels $\geq$ 2 mmol/L), while 5 patients had lactic acidosis (lactate levels $\geq$ 4 mmol/L). Adverse outcomes were reported when lactate levels deviated above 2 mmol/L (Alshiakh, 2023; Godinjak et al., 2017; Kamolz et al., 2005) and numerous studies have demonstrated the prognostic value of lactate ($\geq$ 2 mmol/L) for burn outcomes. It must be emphasized though, that these studies focused on its predictive ability for mortality and did not for inhalation injury (Alshiakh, 2023; Muthukumar et al., 2020; Park et al., 2018; Godinjak et al., 2017). While its role and relationship to inhalation injury had not been the primary focus, indirect relationships could be deduced via correlations between known consequences of inhalation injury, such as cellular hypoxia (Klein et al., 2003; Holm, 2000) and cyanide poisoning (Baud et al., 2002). Hyperlactatemia was also linked to the highly predisposed septic conditions observed in patients with inhalation injuries (Gicheru et al., 2023; Mokline et al., 2017), which potentially provides an explanation for the prevalence of septic shock reported in the TBH cohort with severe injuries.

Similar to the findings in the previous chapter that focused on mortality indicators, the investigated clinical markers for inhalation injury were also interlinked, with one factor potentially exacerbating or perpetuating the other. Consequently, these factors cannot be absolute prognosticators for inhalation injury unless present immediately after incidence, on admission, or shortly thereafter. By this criteria, BICU LOS as a sole parameter should be excluded for consideration since it is a time-dependent parameter. TBSA could potentially provide an accurate indication of the presence and degree of inhalation injury, only if certain factors were also included, *viz*. the duration of exposure, etiology, co-morbidities affecting motility. Additionally, complications may be present on admission only if the patient was referred with unreported incidence and with a prolonged referral window prior to treatment. Lactate levels may be the only clinical marker out of those identified in this study with independent prognostic value since measurements could, immediately or shortly after admission, be determined. Although previously reported research was limited to mortality as the primary predicted outcome, abnormal blood lactate had been established to correlate with hypoxia (Klein et al., 2003; Holm, 2000), known to be part of the inhalation injury-induced sequelae (Foncerrada et al., 2018). This may provide the necessary connection to further investigate this biological parameter in relation to its predictive potential for inhalation injury. Excess lactate was reported to occur within both aerobic and anaerobic conditions. Distinguishing between these events that produce excess lactate should first be considered because aerobic production is an adaptive response to increased metabolic demands (Brooks, 2009; Brooks, 2002; Brooks, 2000) and plays a crucial physiological role in cellular metabolism, while the anaerobic production of lactate occurs in response to hypoxia and has been observed in inhalation injury-related pathologies such sepsis (Ryoo & Kim, 2018; Mokline et al., 2017). Since lactate may not always be indicative of hypoxia or poor perfusion, differentiating between the origin pathways associated with its production would be important in order to relate it as a consequence of inhalation injury. Moreover, changes in lactate levels over time were shown to have better prognostic value in mortality-related reports as opposed to measurements at a single time frame (Mohammed et al., 2023; De Lucas et al., 2020). Thus, of interest in this field to better gauge the relationship between lactate and inhalation injury, a series of measurements over a period of time should be extensively studied. This would allow differentiation between changes in lactate levels from the onset of injury to progressive outcomes, up until patient discharge or mortality. In turn, it would allow the identification of precise cut-off concentrations and time frames for prognostication. Lactate clearance mechanisms should also be considered when validating lactate as a prognostic marker. If exhausted, hyperlactatemia can occur (Ryoo & Kim, 2018), and when fully functional, within 24 hours, survival of burns patient was improved (Kamolz et al., 2005). These mechanisms, therefore, aim to reduce excess lactate and may impact pathological measurements, and thus establishing a timeline for clearance would result in the ability to also determine lactate levels associated with the injury.

Findings based on the patient's history and physical examination have long been relied on for subjective diagnosis of inhalation injury (Kim et al., 2017; Clark, 1992). However, reports have suggested that

these factors alone may not be reliable and lacked association after objective diagnosis using bronchoscopy (Kim et al., 2017; Ching et al., 2015) within 24 hrs post-admission (Palmieri & Klein, 2007). Chemical and thermal mechanisms of the condition was reportedly associated with the observed diagnostic challenges (Dries & Endorf, 2013). This has further been complicated by the heterogenous presentation of the condition (Woodson, 2009; Palmieri & Klein, 2007), with subsequent host response outcomes (Dries & Endorf, 2013). Studies have primarily focused on diagnosing inhalation injury using many associated physical, socioeconomic, and clinical factors. Instead, this study investigated the prognostic potential of the above-mentioned associated factors before the onset of progression. Although prognostication was not clearly met by the identified variables in this study, emphasis should be placed on their collective ability to potentiate the progression of injury after initial assessment, especially in the presence of more than one of the analyzed factors. This would not only aid with selective management and treatment, but also in cases where injury was grossly underscored or undiagnosed. While diagnostic and grading tools have advanced for the improvement of detection, prognostication remains under-researched and requires more investigation. The current diagnostic tools used for burn patients (with the exception of technology such as bronchoscopy) remain fairly subjective. Consequently, studies have broadened diagnostic research to include identifying potential biological markers with greater sensitivity to detection. Moreover, there is a growing interest in the ability of biomarkers for prognostication. Putative biomarkers of interest have ranged from cytokines to larger proteins and also included DNA and RNA easily attained from varying biological sample types such as plasma (Wang et al., 2012), blood (Gallo et al., 2012), and bronchoalveolar lavage fluid (BALF) (Xiao et al., 2018a).

The next chapter will focus on identifying potential RNA biomarkers (microRNAs) in whole blood samples from burn patients admitted to WCPATBC. These biosignatures have been observed in nearly every bodily fluid, with their diagnostic and prognostic potential linked to a plethora of pathological processes and disease pathways. miRNA expression specific to inhalation injury presence, progression, and/or associated complications therefore could present a sensitive, neoteric approach in early identification and subsequent clinical management and support for burn patients with inhalation injury.

CHAPTER SIX
BIOLOGICAL MARKERS AND INHALATION INJURY

10. Blood-based biomarkers for the prognostication of inhalation injury presence

10.1. Overview

Several parameters (i.e. TBSA burns, related complications, BICU LOS, and lactate concentration) assessed in Chapter 5 had statistical suitability as clinical markers; however, these could not solely (independently) be used as a prognosticator or indicator for inhalation injury presence or degree. For instance, TBSA burns cannot consistently indicate the presence of inhalation injury, which can still occur in the absence of flame injuries. Additionally, while metabolic- or respiratory-related consequences can be affiliated with varying lactate levels, pathological accuracy was influenced by early lactate clearance and continuous fluctuations as a result of disease evolution. Similarly, the presence and progress of burns-related infectious complications and BICU LOS, respectively, would require time-course developing periods, which would surpass early diagnostic and prognostication windows. Although unreliable as early indicators, the concurrent presence and progress of any of these factors continue to be indicative of worsening outcomes in burn patients. For the purpose of prognostication, biological markers may be more beneficial due to their disease- and patient-specific functions through pathway modulation. Exemplar biomarkers must be easily detectable and quantifiable, remain stable (Pattarayan et al., 2018), be well-preserved during storage (Liu et al., 2009a; Xi et al., 2007), and be disease-specific (Pattarayan et al., 2018). These characteristics were met by miRNAs, which reportedly also had early predictive potential for numerous pathological diseases (Ghai & Wang, 2016; Cortez & Calin, 2009). The aim of this chapter was to determine differentially expressed (DE) miRNAs in burn patients with mild and severe inhalation injury for potential prognostication thereof. These biomarkers were less invasively attained through blood-based sampling on or shortly after admission in burn patients with existing intravenous lines. Exemplar whole blood samples were then selected based on the clinical data that best potentiated the presence of inhalation injury. The miRNA sequences were retrieved with RNA-sequencing after RNA isolation, aligned to the human genome GRCh38 with sRNA-bench, and counts per million data was generated using Bowtie. EdgeR and DESeq2 in the R statistical program was used to identify the DE miRNAs following normalization methods. The DE miRNAs with $|\log_2 FC| > 1.5$ and adjusted $P < 0.05$ expression values (Song et al., 2022; Robinson et al., 2010; Mestdagh et al., 2009) were considered significant (see Chapter 2: Methodology).

10.2. Results

10.2.1. Small RNA-seq library pooling and mapping

Thirty exemplar samples (15 representing mild and 15 severe inhalation injury) were selected from the 59 total samples based on sequencing pre-requisites, high clinical index of suspicion and additional

clinical parameters utilized by the WCPATBC to indicate inhalation injury presence and degree. Pre-quality (Pre-QC) and quantity analysis, complementary to the Illumina platform specifications, were performed and only samples with RIN >7 and concentration of >2 ng/µl passed for the library preparation. Twenty two samples were accepted for sequencing based on the latter criteria (8 samples representing mild inhalation injury and 14 representing severe) (**Appendix C: Table 16**). The individual small RNA-Seq libraries had a concentration range of 0.1 – 0.5 ng/µl, which were pooled to yield an average library size of 165 bps and a dsDNA concentration of 0.129 ng/µl. Pre-QC metrics demonstrated an average Q(30)-score distribution of 99.1%, Phred scores >30 for both mean and per sequence scores, as well as, expected varying sequence length distributions and sequence duplication levels. Moreover the 3' adapter contaminant present in all samples was removed using the sRNA bench pipeline. Varying GC content per sequence was also observed and corrected via normalization downstream. Overall, these results demonstrated successful library production for the 22 exemplars (n=8 mild and n=14 severe injury samples) as denoted by the aforementioned satisfactory pre-QC results. FastQC files were generated, and the adapters of the raw sample reads (between 316861 and 22138349) were trimmed. Only clean reads that were 10 nt-length sequences with Phred scores >20 were used for downstream analysis. The subsequent clean reads obtained ranged between 253453 and 8 million for mild injury, and the range for severe injury was between 474833 and 19 million. Following mapping to the human genome GRCh38 and re-alignment to human miRbase (v22) data, the average percentage of clean reads that were successfully mapped was 97.29%, ranging between 96.34% and 98.33% (**Appendix C: Table 17**).

10.2.2. Differentially expressed miRNAs in mild and severe inhalation injury using EdgeR and DESeq2

DE miRNAs were determined with EdgeR and DESeq2 after alignment and mapping to the human genome. A cumulative 335 miRNAs aligned for mild and severe inhalation injury using both tools. Of these, 60 DE miRNAs (30 up- and 30 down-regulated) were obtained with EdgeR (**Figure 5A & 6A**, **Annexure C: Table 18**), 67 DE miRNAs (39 up-regulated and 28 down-regulated) were observed with DeSeq2 (**Figure 5A & 6B**, **Annexure C: Table 19**) and 57 overlapped between the two modalities (**Annexure C: Table 20)**. DE miRNAs in mild injury samples were 30 and 28 for EdgeR and DESeq2, respectively; with an overlap of 27 between the outputs from the two tools (**Figure 5B**). DE miRNAs in severe injury were 30 and 39 for EdgeR and DESeq2, respectively; with an overlap of 30 (**Figure 5C**). All DE miRNA in mild injury were down-regulated, while only up-regulated DE miRNA were observed for severe injury. In order to select the DE miRNAs for target gene analysis, |log2 FC|>1.5 and Padj.<0.05 were used as cut-off values. With these criteria in mind, 10 DE miRNAs were observed with EdgeR, which also overlapped with those from DESeq2, that in turn yielded 14 DE miRNAs in total (**Figure 5D**).

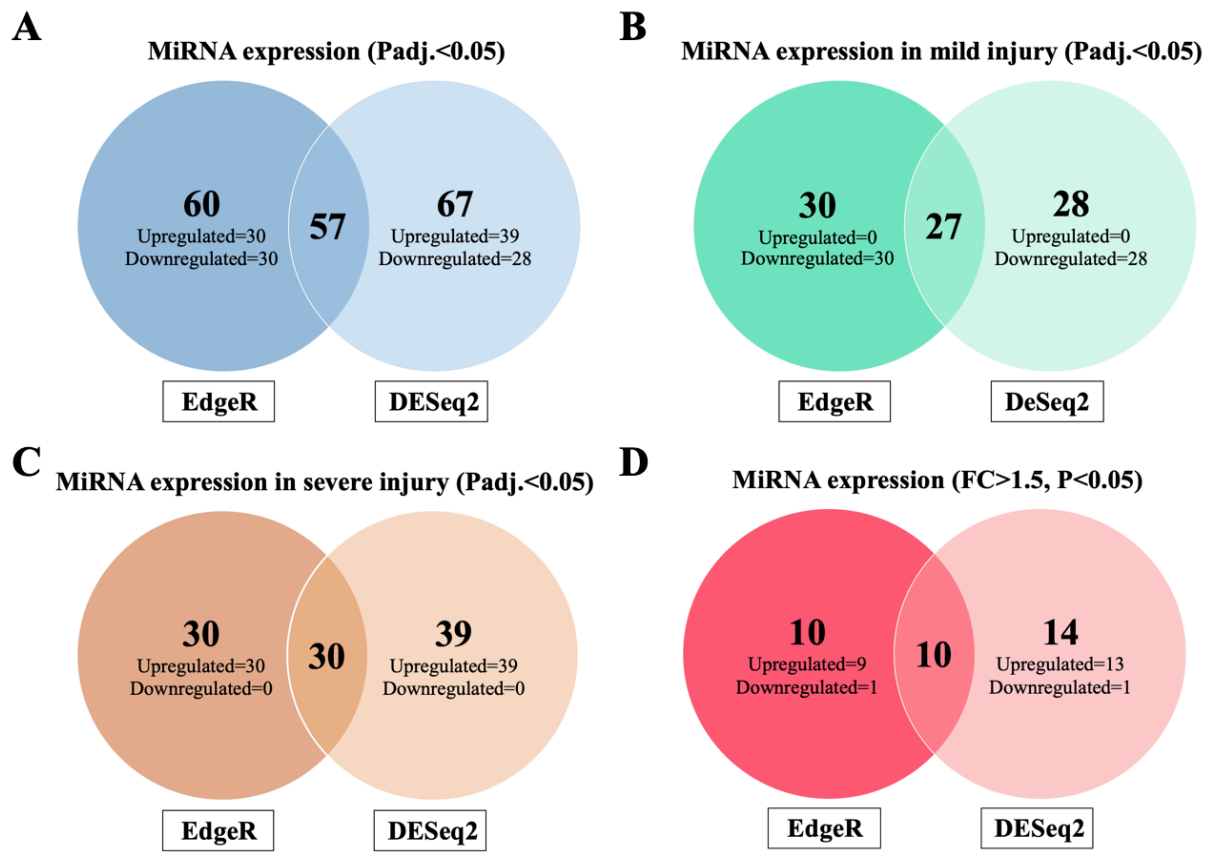


Figure 5: DE miRNA detected with EdgeR and DESeq2 (Padj.<0.05): **A**: DE miRNAs detected using EdgeR and DESeq2. **B**: DE miRNAs in mild inhalation injury. **C**: DE miRNAs in severe inhalation injury. **D**: |log2 FC|>1.5 DE miRNAs (DE – differentially expressed, Padj. – P-adjusted, FC – fold change).

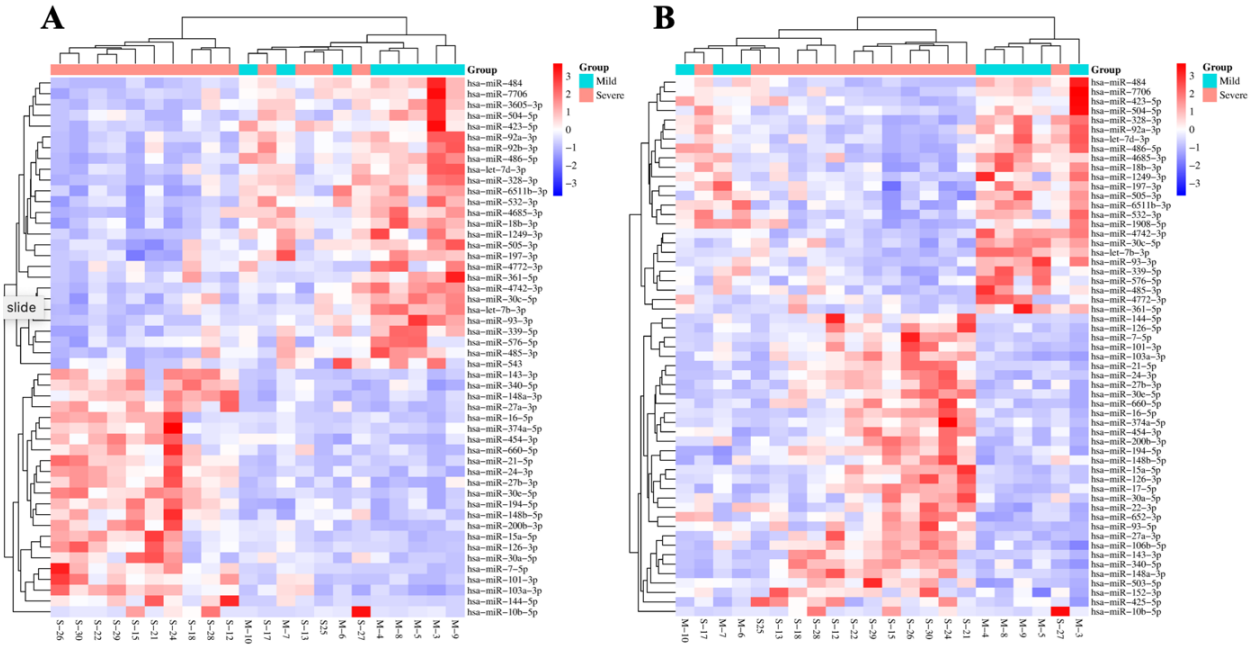


Figure 6: Significant DE miRNAs: A: Significant DE miRNAs obtained with EdgeR (Padj.<0.05). **B**: Significant DE miRNAs obtained with DESeq2 (Padj.<0.05). Up- and down-regulation is represented by red and blue-coloured blocks, respectively, while the colour intensity thereof has a direct relationship with expression levels DE – differentially expressed, Padj. – P-adjusted.).

10.2.3. Differentially expressed miRNAs in mild and severe inhalation injury meeting cut-off values

The ten intersecting DE miRNAs with |log2 FC|>1.5, Padj.<0.05 were considered for target gene and pathway enrichment analysis (**Figure 7A and B**). Those not overlapping (n=4: miR-101-3p, -126-3p, -7-5p, 30e-5p) had FCs slightly below or just made the target cut-off value (1.45, 1.49, 1.49, 1.50, respectively) which also supported the basis for omission from further analysis (**Figure 7B**). One DE miRNA (miR-504-5p) was observed in mild inhalation injury, while the remaining 9 (miR-143-3p, -200b-3p, -148b-5p, -10b-5p, -30a-5p, -15a-5p, -374a-5p, -21-5p, -144-5p) were all significant for severe inhalation injury (**Figure 8A and B**). Moreover, these 10 DE miRNAs had near identical mean counts and FCs with both tools, while the single DE miRNA in mild injury was down-regulated, and the remaining 9 attributed to severe injury were all up-regulated (**Table 7**).

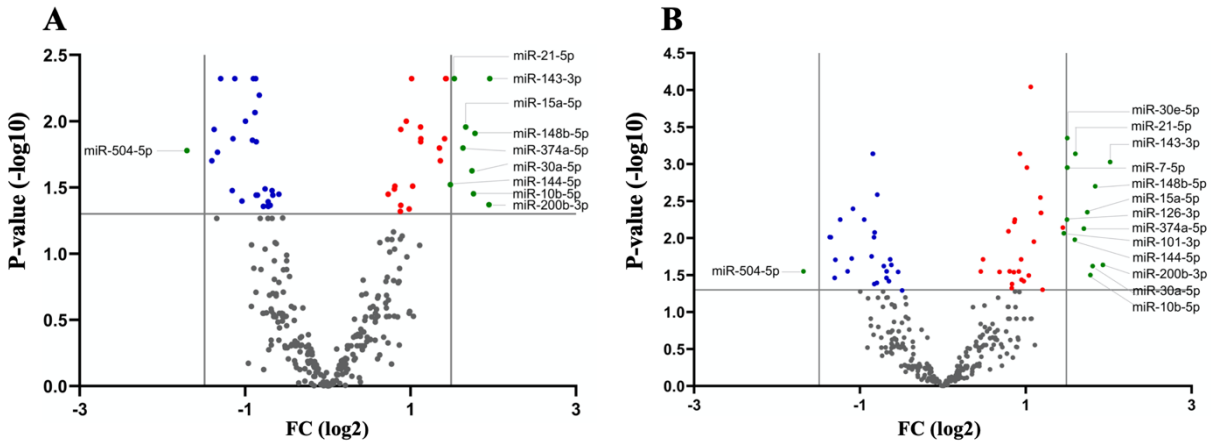


Figure 7: Significant DE miRNAs with |log2 FC|>1.5 and Padj.<0.05 expression values: **A**: Significant DE miRNAs detected by EdgeR incorporating the cut-off criteria. **B**: Significant DE miRNAs detected by DESeq2 incorporating the cut-off criteria. Blue plots denoted down-regulated DE miRNAs, red plots denoted up-regulated DE miRNAs and green plots illustrated DE miRNAs that met the cut-off criteria values. Cut-off indicators were represented by vertical lines for FC values and the horizontal line at >1.3 -log10 for Padj.-values (DE – differentially expressed, FC – fold change, Padj. – P-adjusted.).

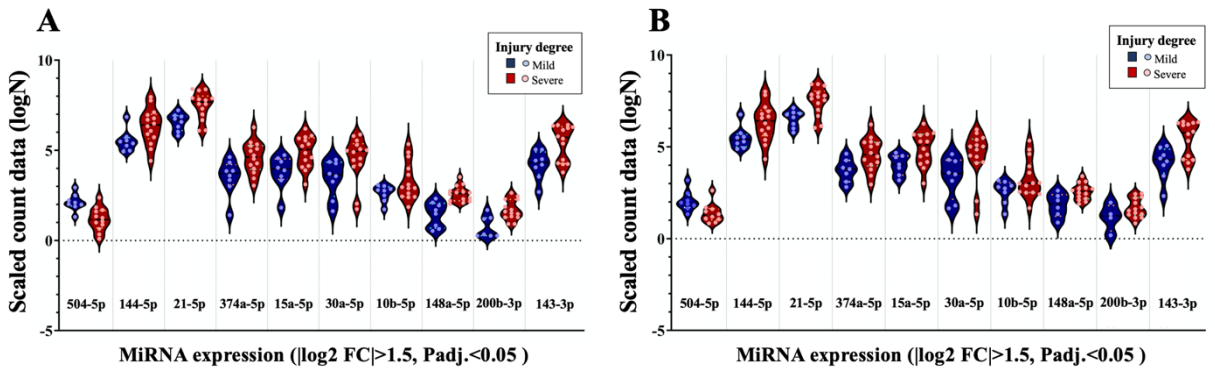


Figure 8: Significant DE miRNAs of mild and severe inhalation injury with |log2 FC|>1.5 and Padj.<0.05 expression values: **A**: DE miRNAs detected with EdgeR and **B**: DE miRNAs detected with DESeq2. The violin plots represented the mean counts per million, the contained dots denoted the individual counts per sample, mild

inhalation injury was represented by blue plots and severe injury by the red plots (DE – differentially expressed, FC – fold change, Padj. – P-adjusted.).

Table 7: Up- and down-regulated DE miRNAs detected with EdgeR and DESeq2 for inhalation injury degree that meet the cut-off criteria (|log2 FC|>1.5 and Padj.<0.05)

MiRNA (ID: *Accession #*)	**Mean (Count/million)**		**FC (Log2)**		**P-value (Adjusted)**		**Regulation (Up/down)**	**Degree (Mild/severe)**
	EdgeR	*DESeq2*	*EdgeR*	*DESeq2*	*EdgeR*	*DESeq2*		
miR-504-5p: *MIMAT0002875*	8.2	8.1	-1.7	-1.7	0.02	0.03	Down	Mild
miR-144-5p: *MIMAT0004600*	853.4	876.2	1.5	1.6	0.03	0.01	Up	Severe
miR-21-5p: *MIMAT0000076*	2247.1	2255.6	1.5	1.6	0.00	0.00	Up	Severe
miR-374a-5p: *MIMAT0000727*	138.8	137.9	1.6	1.7	0.01	0.01	Up	Severe
miR-15a-5p: *MIMAT0000068*	183.8	185.7	1.7	1.7	0.01	0.00	Up	Severe
miR-30a-5p: *MIMAT0000087*	145.9	146.6	1.7	1.8	0.02	0.02	Up	Severe
miR-10b-5p: *MIMAT0000254*	47.1	46.0	1.8	1.8	0.03	0.03	Up	Severe
miR-148b-5p: *MIMAT0004699*	13.5	13.3	1.8	1.8	0.01	0.00	Up	Severe
miR-200b-3p: *MIMAT0000318*	5.2	5.0	1.9	1.9	0.04	0.02	Up	Severe
miR-143-3p: *MIMAT0000435*	317.9	315.2	2.0	2.0	0.00	0.00	Up	Severe

DE – differentially expressed, FC – fold change, Padj. – P-adjusted.

10.2.4. The hub miRNAs in the miRNA-mRNA networks of inhalation injury

The up-regulated, down-regulated DE miRNAs and the combination of the two groups were analyzed with the miRNet database to determine their importance in the miRNA-mRNA network by degree of connectivity. The degree values of the miRNAs (nodes) represent the amount of target genes it has connections with. The nodes with higher degrees were important in the network since malfunctioning thereof would influence all the connections. For miRNA groups; miR-30a-5p, miR-15a-5p and miR-21-5p targeted the most genes with 734, 717 and 612 degrees, respectively (**Table 8**).

Table 8: Degrees and betweenness of the 10 DE miRNAs with target genes in the mRNA network

MiRNA ID: *Accession #*	**Regulation** *(Up/down)*	**Target gene network topology**	
		Degree	*Between*
miR-504-5p: *MIMAT0002875*	Down	108	175456
miR-144-5p: *MIMAT0004600*	Up	36	63782.52
miR-21-5p: *MIMAT0000076*	Up	612	1243746
miR-374a-5p: *MIMAT0000727*	Up	282	533788.9
miR-15a-5p: *MIMAT0000068*	Up	717	145890
miR-30a-5p: *MIMAT0000087*	Up	734	1514183
miR-10b-5p: *MIMAT0000254*	Up	323	644612
miR-148b-5p: *MIMAT0004699*	Up	52	99717.31

miR-200b-3p: *MIMAT0000318*	Up	186	335533.2
miR-143-3p: *MIMAT0000435*	Up	228	468866.7

DE – differentially expressed.

10.3. Discussion

Early detection of inhalation injury continues to be an unmet need that would improve burns-related outcomes and reduce mortality incidence. Diagnostic tools have been able to identify certain aspects of the injury, and prognosticating modalities have demonstrated promise in differentiating degree; however, numerous challenges exist that particularly implicate resource-limited clinical settings and subsequent management. In Chapter 5, burns-related variables were investigated as potential prognostic markers; however, these were better suited for identifying disease progression. In this chapter, biological markers (miRNAs) were instead assessed as potential prognosticators based on the premise that these biomarkers were differentially expressed in the exemplar samples that best potentiated for the presence of inhalation injury. The samples represented two inhalation injury degrees, mild and severe, of which potential DE miRNAs with the latter degree were of particular interest since increasing degree significantly correlated with and predicted mortality in the burn cohort of this study (See Chapter 4). Thus, after aligning sequences to the human genome, the differentially expressed miRNAs were compared between the mild and severe injury samples. Ten miRNAs overlapped between the results obtained with EdgeR and DESeq2 and met the threshold cut-off values for DE identification. Only 1 (miR-504-5p) was significant for mild injury and down-regulated, whereas the remaining 9 (miR-143-3p, -200b-3p, -148b-5p, -10b-5p, -30a-5p, -15a-5p, -374a-5p, -21-5p, and -144-5p) were all up-regulated in the severe injury samples. Although miRNA profiles in burn injuries have been investigated, it was to a much lesser extent compared to oncological studies; and of the very few studies assessing these putative markers for inhalation injury, different species (models) and methods were utilized. This was therefore the first study identifying a potential panel of putative miRNAs in human whole blood samples for inhalation injury degree. Most of the studies addressed miRNA expression in cutaneous burns (skin tissue) utilizing either rat (Zhou et al., 2016) or mouse (Zhang et al., 2020a; Lyu et al., 2018; Morris et al., 2017) models. Scalding was the main mode of burn induction; however, miRNAs in localized radiation injury were also investigated (Ancel et al., 2024). In addition, secondary outcomes of burns, such as hyperglycemia (Zhang et al., 2017b), insulin resistance (Karolina et al., 2011; Ryu et al., 2011), acute kidney injury (AKI) (Wang et al., 2023a; Petejova et al., 2022), sepsis (Xu et al., 2022), hypertrophic scarring (HS) (Mu et al., 2016; Zhu et al., 2014), and skeletal muscle atrophy (Yu et al., 2016), were also topics of interest in relation to dysregulated miRNA levels and their post-transcriptional consequences. At least half of the upregulated DE miRNAs in this study were also up-regulated in the aforementioned reported conditions. MiR-15a-5p and miR-144-5p were up-regulated in deep partial thickness burn skin (Liang et al., 2012), whereas miR-200b-3p was up-regulated in HS tissue (Zhou et al., 2015; Li et al., 2014) along with miR-21-5p (Zhou et al., 2015; Zhu et al., 2014). Elevated levels of miR-148b-5p were observed in localized radiation injury (Ancel et al., 2024), while a study observing

miRNA profiles in fire-fighter blood samples demonstrated elevated miR-374a-5p levels (Jeong et al., 2018). Conflicting results were also reported where the up-regulated miR-200b-3p, -143-3p, -148b-5p, -15a-5p, and -21-5p in this study were down-regulated in deep partial thickness burn skin (Liang et al., 2012), HS tissue (Mu et al., 2016), skeletal muscle atrophy (Haijun et al., 2015), sepsis-induced AKI (Petejova et al., 2022), and sepsis (Xu et al., 2022); respectively.

Although none of these studies factored in the potential concurrent presence of inhalation injury or focused solely on the condition itself, the identical expression of the miRNAs with this study may have resulted from overlapping cellular and molecular mechanisms that stem from non-specific immune or inflammatory responses after the initial insult, despite the condition or injury being different. Inhalation injury and miRNA expression have been analyzed in mice (Jiang et al., 2022; Zhang et al., 2020c) and rats (Xiao et al., 2018) after smoke exposure in an enclosed holding for specific durations. Either lung tissue or BALF was assessed using RT-qPCR or microarray analysis. Since the models and methods of the latter reports differed from this study, variations in miRNA expression were expected. The expression of miRNA-200b-5p in the BALF of smoke-induced rat models (Xiao et al., 2018) was consistent in this study; however, it comprised the bottom 3 miRNAs that had the least degree values (connections) with target genes. MiRNAs with the most connections (higher degrees) in a network were considered as the most important "hubs" (Chang & Xia, 2023; Chang et al., 2020; Zhu et al., 2007) since their dysregulation would impact all the genes that they target (Li et al., 2018), which in turn would alter the control and balance of the entire network, causing disease. The potential impact of these influential miRNAs was further explored in literature with particular emphasis on their role in inflammatory responses and apoptosis. The release of immune and inflammatory mediators post-inhalation injury would not only determine the extent of the inflammation (Rehberg et al., 2009) that manifests clinically, but with inhalation injury-related complications, such as hypoxia (Coimbra-Costa et al., 2017) and pneumonia-induced sepsis (Lobo et al., 2005), these mediators were shown to activate apoptosis through oxidative stress (Yang et al., 2020; Park et al., 2009). Apoptosis was one of the important events in the development of numerous pulmonary conditions such as chronic obstructive pulmonary disease (Demedts et al., 2006) and pulmonary fibrosis (Morimoto et al., 2012). Interestingly, it was the prolonged inflammation caused by apoptosis that played a role in the pathogenesis of the latter conditions. The exacerbated inflammation was a reported outcome of excessive apoptotic cell function, or failure to inhibit this activity, that elevated pro-inflammatory mediator release (Juncadella et al., 2013; Vandivier et al., 2002). The interactions between inflammatory and apoptotic mechanisms may not only overlap but could also be crucial in the onset and progress of inhalation injury. Therefore, the primary focus was on the potential relationship between these events and the "hub"/influential miRNAs in this study.

The miRNAs with the highest degrees (in descending order) were miR-30a-5p, -15a-5p, and -21-5p; and were all up-regulated in the samples representing severe inhalation injury. Several studies observed the

abhorrent expression of these miRNAs in disease governed by inflammation. The overexpression of miR-30a-5p had a pro-inflammatory impact in periodontitis (Liu et al., 2021b) and Alzheimer's disease (AD) (Sun et al., 2022), of which the latter was induced by the production of amyloid-beta peptide triggering oxidative stress and inflammatory reactions (Zhang et al., 2021b). Liu et al, (2021) conclusively proved this role by introducing a miR-30a-5p inhibitor, which reduced the initial inflammatory effect in periodontitis. Although these studies demonstrated the pro-inflammatory effect of miR-30a-5p, there were comparatively more reports demonstrating its anti-inflammatory properties. ALI/ARDS-related inflammation was simulated by lipopolysaccharide (LPS)-induced effects in murine lung (Li et al., 2021b). LPS was reported as an important ALI risk factor (Fu et al., 2014; Joh et al., 2012) causing inflammation by activating cells to release inflammatory cytokines (Kim et al., 2013; Uda et al., 2013) that exacerbate lung injury. Following LPS introduction, reduced miR-30a-5p levels and cell viability in lung and peripheral blood was observed, along with elevated pro-inflammatory cytokines (such as IL-1β, IL-6, and TNF-α). The isolated lung cells were then transfected with miRNA-30a-5p simulators, which then ameliorated the lipopolysaccharide-induced effects on cell cycle and inflammation (Li et al., 2021b). The anti-inflammatory effect of miR-30a-5p overexpression was also observed in conditions where inflammation was the hallmark of the adverse outcomes. In atherosclerotic murine models, anti-inflammatory outcomes (induced by IL-1β, IL-6, and TNF-α) and lipid uptake (of low-density lipoproteins, triglycerides, and total cholesterol) were beneficially modulated by the up-regulation of miR-30a-5p, along with the concurrent reduction in pro-inflammatory effects (induced by IL-10 and TNF-β) (Song et al., 2021). In obese mice, adipocytes were protected from the pro-inflammatory cytokine IFN-γ that decreased peripheral insulin sensitivity when miRNA-30a-5p was overexpressed (Koh et al., 2018). The role of miR-30a-5p in LPS-induced neuroinflammation of murine microglial cells was investigated by two studies. The underlying LPS-stimulated mechanisms, which included the activation of TNF-α, IL-6, NO, iNOS and NF-κB, were all attenuated with the introduction of miR-30a-5p (Kim & Yang, 2022). Moreover, miRNA-30a-5p affected the neuroinflammation-driven pathways that were mediated by iNOS, IL-1β, TNF-α, IL-6 secretion, and ROS by regulating and suppressing phosphorylation of these pathways that typically caused the inflammation (Choi et al., 2022). The same pathological trend was observed with miR-15a-5p and miR-21-5p in that inflammation could either be suppressed or promoted depending on the condition, cell type, and expression levels.

Overexpression of miR-15a-5p resulted in inflammatory consequences attributed to the pathology of LPL-induced sepsis (Lou & Huang, 2020), traumatic haemorrhagic shock-induced ALI (Zhou et al., 2020), and periodontitis (Costantini et al., 2023; Luan et al., 2018). Elevated levels of IL-1β, IL-6 and TNF-α (Lou & Huang, 2020; Zhou et al., 2020; Costantini et al., 2023; Luan et al., 2018) were the causative pro-inflammatory mediators resulting in the observed inflammation. MiR-15a-5p's regulatory function in inflammation was supported when the elevated release of pro-inflammatory mediators and the activity of the suspected nuclear factor kappa (NF-κ) signaling pathway involved were prevented after miR-15a-5p inhibitors were introduced into the models (Lou & Huang, 2020; Zhou et al., 2020).

The anti-inflammatory properties of miR-15a-5p were also demonstrated in the following studies: (1) in atherosclerotic arterial tissues of rats, which reduced fat storage and improved vasculature, endothelial cell function, and lipid metabolism (Liu et al., 2019), (2) in human and murine atherosclerotic models, which decreased NF-κB (González-López et al., 2023), and (3) in oxygen-induced retinopathy (to assess retina angiogenesis) and laser-induced choroidal neovascularization (to assess choroid angiogenesis), which reduced neovascularization, promoted vascular growth, and decreased inflammation and fibrosis (Li et al., 2024a). Similarly, miR-21-5p could also promote and inhibit inflammation, with cell- and condition-specific effects. Most of the studies reported that increased miR-21-5p had pro-inflammatory outcomes in response to numerous conditions such as chronic rhinosinusitis with nasal polyps (Luan et al., 2022) or nasal polyps alone (Liu et al., 2021a), psoriatic arthritis (Machhar et al., 2019), LPS-induced cardiovascular damage (Xue et al., 2021), LPS-induced ALI (Zeng et al., 2024; Yang et al., 2019; Zhu et al., 2018), and degenerative cervical myelopathy (Laliberte et al., 2021). The aggravated inflammation of these conditions was once again attributed to the presence of elevated proinflammatory cytokines/chemokines such as TNF-α (Zeng et al., 2024; Xue et al., 2021; Zhu et al., 2018), IL-1β, IL-6, IL-8, and IL-25 (Liu et al., 2021a; Zeng et al., 2024; Xue et al., 2021; Zhu et al., 2018) and the concurrent reduction in the anti-inflammatory cytokine, IL-10 (Liu et al., 2021a; Zhu et al., 2018). Additionally, the pro-inflammatory implications of miR-21-5p were also reported in intestinal inflammation (Shi et al., 2013) and allergen-induced allergic airway inflammation (Lu et al., 2009) as well as various cell types after LPS-stimulated injury such as lung fibroblasts (Li et al., 2021a), mouse lung microvascular endothelial cells (Jiang et al., 2020), and human alveolar epithelial cells (Ge et al., 2020). However, anti-inflammatory effects related to miR-21-5p were also observed in LPS-affected murine macrophage cell lines (Yang et al., 2019), lung macrophages (Zhu et al., 2014), human pulmonary epithelial cell lines (Zeng et al., 2024), and mouse lung tissue (Moschos et al., 2007). The regulatory role of miR-21-5p in inflammation was confirmed by countering the inflammatory-related effects in different diseased models with mimics (Zeng et al., 2024; Xue et al., 2021; Zhu et al., 2018), inhibitors (Yang et al., 2019), or making use of knockout models (Luan et al., 2022; Laliberte et al., 2021; Jansing et al., 2020). Interestingly, the mimics were incorporated to overexpress miR-21-5p in models where the pathological condition itself already up-regulated the marker. A protective role was attributed to miR-21-5p in these cases. Most of the studies reported upregulated miR-21-5p levels in response to a diseased state; however, Chen et al, (2020) observed reduced miR-21-5p levels in response to obstructive sleep apnoea that resulted in elevated TNF-α, while the addition of a mimic suppressed the TNF-α levels (Chen et al., 2020). The varying miRNA levels observed in literature would understandably be different considering models, samples, and disease. However, the presence of these hub miRNAs has strongly and consistently been involved in regulating the balance between pro- and anti-inflammatory responses throughout literature and, of particular interest, in diseases or conditions that were either similar to inhalation injury outcomes or were sequelae thereof. Therefore, these hub miRNAs were also postulated to play an important role in the development of the inflammation observed with inhalation injury. However, it should be noted that most of the remaining markers, i.e. miR-143-

3p (Wang et al., 2020; Yang et al., 2018), -200b-3p (Wei et al., 2024; Lin et al., 2020), -148b-5p (Datta et al., 2023; Miscianinov et al., 2018), -10b-5p (Dash et al., 2024; Li et al., 2024b), -374a-5p (Ma et al., 2024; Perez-Sanchez et al., 2022), and -144-5p (Wu et al., 2023; Sundquist et al., 2021), were also implicated in diseases that focused on inflammatory outcomes. This would highlight the potential of these miRNAs to influence inflammatory outcomes in inhalation injury as well, potentially to a lesser degree compared to hub miRNAs or perhaps as a functional cluster incorporating the hub miRNAs as well.

Similar to how miRNA influenced inflammatory responses, miR-30a-5p, -15a-5p, and -21-5p also had a bi-functional role in apoptosis by modulating both pro- and/or anti-apoptotic responses to either enhance or inhibit disease pathogenesis, respectively. These outcomes also varied according to the cell type and condition being investigated. The altered expression of miR-30a-5p was observed in several diseases, both seemingly unrelated and potentially related to inhalation injury. Up-regulated expression of miR-30a-5p resulted in apoptosis that contributed to AD (Sun et al., 2022), while several studies that focused on the pathogenesis of hypoxic-related or -induced conditions (such as hypoxia-induced pulmonary arterial hypertension (Tan et al., 2019), myocardial hypoxia/reoxygenation (Lv et al., 2021), and hypoxia-induced acute myocardial ischemia (Liang et al., 2024) also reported on the impact of apoptosis as a consequence of miR-30a-5p overexpression. Conversely, LPS-induced ALI/ARDS in A549 lung epithelial cell lines (Li et al., 2021b), renal ischemia/reperfusion injury in HK-2 proximal tubular kidney cells (Fang et al., 2021), and cypermethrin-induced reproductive damage in TM4 Sertoli cells (Wang et al., 2023b) resulted in reduced miR-30a-5p expression that still induced apoptosis. Regardless of the varying expression levels and models, these studies went on to confirm the connection between miR-30a-5p and apoptosis induction by transfecting the respective pathogenic models with miR-30a-5p mimics and/or inhibitors, which reversed the initial resultant apoptosis. It's not surprising that miR-30a-5p would be focused on as ideal therapeutic targets in disease because of its reported regulatory role in apoptosis. It is even more unsurprising that the interest in targeting the miR-30a family had grown exponentially in cancer-related studies due to its ability to amplify apoptosis and reduce cell proliferation (Jia et al., 2013; Wang et al., 2013). MiR-30a-5p has been observed in numerous reports where its down-regulation contributed to cancer metastasis, and its overexpression enhanced apoptosis and reduced cell growth that was necessary to counter the tumour growth (Quan et al., 2019; Liu et al., 2018; Li et al., 2016b; Wei et al., 2016).

As observed with miR-30a-5p, up-regulated miR-15a-5p levels were observed in the bone marrow stem cells involved with femoral head necrosis (Zhang et al., 2019a), poor ovarian response that negatively impacted reproductive outcomes (Zhang et al., 2017a), pulmonary arterial hypertension (Zhang et al., 2019b), and LPS-induced ALI (Zhu et al., 2023). The upregulation was not only a consequence of the condition that resulted in apoptosis, but these studies demonstrated that overexpression of miR-15a-5p after its initial increase, exacerbated apoptosis (Zhang et al., 2019a; Zhang et al., 2019b). Zhu et al,

(2023) further demonstrated that the initial miR-15a-5p levels in LPS-induced ALI were reduced when treated with protein kinase C-alpha treatment (known to have a protective role in sepsis-induced ALI), which concurrently repressed apoptosis and inflammatory mediators. Conversely, overexpressed miR-15a-5p could also beneficially reduce apoptosis in studies that originally observed reduced levels with temporal lobe epilepsy (Li et al., 2020), LPS-treated lung cells (Hong et al., 2021), and sepsis-induced ALI (Lin et al., 2025). Varying expression levels of the third highest ranked miRNA, miR-21-5p, was also observed in various diseases. Up-regulated levels were attributed to COPD (Kim et al., 2021), ulcerative colitis (Lu et al., 2019), and Clostridium perfringens toxin infection (Gao et al., 2021). Lu et al, (2019) observed reduced apoptosis along with IL-6 and TNF-α once their ulcerative colitis-induced models were treated with miR-21-5p inhibitors (Lu et al., 2019), while further overexpression with miR-21-5p with mimics in the Clostridium perfringens toxin infection study also reduced apoptotic rates, which was reversed when inhibitors were applied (Gao et al., 2021). By comparison, down-regulation of miR-21-5p was reported in ischemic stroke (Zhan et al., 2023), LPS-induced white matter injury (Zhang et al., 2022), rheumatoid arthritis (Yan et al., 2019a), hyperoxic ALI (Qin et al., 2019), and sepsis-induced AKI (Zhang et al., 2021c). Its role in apoptosis was confirmed by the addition of mimics or agonists improving the apoptotic outcomes in all the above-mentioned studies. It should also be noted that the altered expression of the remaining miRNAs in the panel viz. miR-504-5p (Hu et al., 2021; Cao et al., 2017), -143-3p (Zhao et al., 2022; Yang et al., 2018), -200b-3p (Zhang et al., 2023; Zhang et al., 2021a), -148b-5p (Zhu et al., 2024; López-Sánchez et al., 2022), -10b-5p (Liu et al., 2024; Wu et al., 2019), -374a-5p (Wang & Mei, 2023; Umehara et al., 2020), and -144-5p (Wu et al., 2023; Fu et al., 2019); also reportedly played a role in regulating apoptosis in varying diseases. The potential for these individual miRNAs to also influence apoptosis and subsequent pathology is once again highlighted. Their contribution, either individually or collectively, in inhalation injury pathology through apoptotic mechanisms cannot conclusively be omitted, and when considering the regulatory ability in overlapping pathways, further studies would be required to conclusively determine their functional potency over the more influential miRNAs in this study.

Variable expression levels in previous studies that addressed similar conditions to this study, such as those of miR-15a-5p in LPS-induced ALI versus LPS-induced sepsis, or with miR-21-5p in COPD versus hyperoxic ALI, corroborated the expected outcomes that the observed miRNA expression was dependent and influenced by differences in the sample types, the methodology used, and the condition (pathology) investigated. Moreover, the altered expression of miR-30a-5p, miR-15a-5p (Lin et al., 2025; Zhu et al., 2023), and miR-21-5p (Zhang et al., 2021c; Lu et al., 2019) on apoptosis was often investigated alongside the impact on inflammatory mediators. Investigations into both events, simultaneously, were suggestive of their impact in diseases that were closely related to inhalation injury outcomes and complications. It was evident that these hub miRNAs have the potential to be key players in the pathogenesis of any disease (including inhalation injury), particularly since studies have demonstrated their multifunctional implications by observing their expression both when the conditions

were induced and when the outcomes were manipulated with mimics or inhibitors. Based on this, it would then be imperative to consider that the observed abhorrent expression could occur as a result of the injury or due to a potential protective function by responding to the injury. Identifying the expression for these responses will potentially further narrow down the ideal markers for inhalation injury identification. However, because the samples were collected on or shortly after admission (even with transferral time accounted for) and before severe inflammation settled in, it is plausible to suggest that the panel of miRNAs in this study was a reflection of the insult itself and not as compensation after reacting to the injury. Moreover, since miRNAs were able to interact with each other and numerous other mediators that affects expression as well, differentiating between the initial and secondary miRNAs may also more efficiently represent immediate injury manifestation. MiRNAs are able to interact directly or indirectly with other miRNAs to influence the expression, which can affect the biogenesis and subsequent synthesis (Hill & Tran, 2022; Tang et al., 2012). As part of direct interactions, miRNAs can complementarily bind to another to influence stability, gene targeting, and regulation (Hill & Tran, 2022; Park et al., 2017; Guo et al., 2012; Lai et al., 2004). More interesting is the 'miRNA hierarchy' concept, which describes an initial miRNA group's ability for widespread post-transcriptional regulation, which in turn introduces secondary miRNA to continue the cascade of post-transcriptional regulation (Tang et al., 2012). This may even be a reason for the efficient functioning of a cluster compared to a single miRNA (Niu et al., 2023). Indirect interactions include secondary transcriptional control mediated by transcription (Hill & Tran, 2022; Hill & Tran, 2021; van Rooij et al., 2009), methylation (Song et al., 2015), promoter (Hill & Tran, 2021), repressor (van Rooij et al., 2009), and epigenetic factors (Hill & Tran, 2022; Hill & Tran, 2021) that mediate other miRNAs through pathways control. The formation of feedback loops with inflammatory pathways is another form of indirect interactions where specific miRNAs regulate the activity of inflammatory mediators that, in turn, can regulate the activity and expression of other miRNAs (Su et al., 2016). The dynamic expression patterns exhibited by miRNAs change due to these influences, but also in response to different stress types (Naqvi et al., 2022) and rapidly over time (Mi et al., 2021; O'Brien et al., 2018). Profiles have changed at intervals as short as 2 hours, extending over and up until 24 hours (Diener et al., 2020; Li et al., 2016a). The time-sensitive nature of expression responses would therefore suggest that the identification of the initial miRNAs better depicts the prognosis of any condition; however, the secondary miRNAs stimulated by the initial group or through indirect mechanisms would also not be far off provided the window in passing was not extensive. This once again would allude to the ability of miRNAs to function on their own or in clusters in altering cell functions and lineage (Hill & Tran, 2022) observed in pathologies, but also stress their ability to be expressed at early stages of disease for early detection of that disease. The development of these clinical outcomes, however, is executed through the interconnected and multi-layered nature of miRNA regulatory networks. The first step in the regulatory network would be the modulation of target genes (mRNAs) by the miRNAs. Subsequently, the proteins translated from the respective mRNAs can trigger pathways that are specific for the observed inflammation and apoptosis. This chapter provided a potential panel of miRNAs for inhalation injury

prognostication based on the previous findings that demonstrated their key roles in inflammatory and apoptotic responses, and in conditions closely related to inhalation injury. The next chapter will address target genes and their potential pathways that were modulated by these miRNAs and that were relative to inflammation and apoptosis as hallmarks in inhalation injury.

CHAPTER SEVEN
BIOLOGICAL MARKER PATHWAYS AND INHALATION INJURY

11. Target genes and pathways of blood-based biomarkers for the prognostication of inhalation injury presence

11.1. Overview

MiRNAs have been located in nearly all bodily tissues and remain pivotal modulators in diverse developmental processes. Given its accessibility and regulatory function, aberrant expression of miRNA profiles can be tied to nearly any existing pathological condition. In chapter 6, the results from 2 datasets obtained with separate genomic tools (EdgeR and DESeq2) were combined, and the overlapping miRNA with $|\log2\ FC|>1.5$ and adjusted $P<0.05$ expression values (Song et al., 2022; Robinson et al., 2010; Mestdagh et al., 2009) were considered differentially expressed (DE) for mild and severe inhalation injury samples. Ten overlapping DE miRNAs were identified and comprised 1 down-regulated miRNA for mild injury and 9 up-regulated for severe inhalation injury. According to the literature discussed in the related chapter, the identified miRNAs were involved in processes connected to several burns-related pathologies (Wang et al., 2023a; Xu et al., 2022; Mu et al., 2016; Zhu et al., 2014; Liang et al., 2012). It is therefore reasonable to speculate that these miRNAs may also play important roles in the initiation and progress of inhalation injury. This plausibility is further supported by studies not only demonstrating the connection to inhalation injury sequelae, such as sepsis (Xu et al., 2022) and ALI/ARDS (Li et al., 2021b), but also to the pathological hallmarks thereof (inflammation (Lou & Huang, 2020) and apoptosis (Li et al., 2021b). In order to determine whether the panel of 10 miRNAs could contribute to the development and/or progress of inhalation injury, it is important to identify the potential underlying regulatory functions thereof through implications on target genes and subsequent pathway involvement. We hypothesized that these miRNAs would target mRNAs involved in inflammatory and apoptotic pathways, which would directly relate to inhalation injury pathologies. Therefore, the aim of this section was to determine the target and hub genes as well as the affected biological processes regulated by the miRNA panel in order to elucidate potential connections to inhalation injury. To achieve this, the following methodology was employed: *(1)* miRNet to determine the target genes, *(2)* STRING and Cytoscape to visualize and analyze protein-protein interactions, *(3)* Cytoscape's MCODE and cytoHubba plug-ins for the top vital module/cluster and hub gene identification, respectively, and *(5)* GO, KEGG, Reactome, and Panther assessed the functional pathway enrichment involvement (see Chapter 2: Methodology).

11.2. Results

11.2.1. Target genes of differentially expressed miRNAs in miRNA-mRNA networks

The biological outcomes of miRNAs occur primarily by directly targeting mRNA, thus these potential target genes were also determined using the miRNet database. Input with the 9 up-regulated DE miRNAs resulted in mRNA network with 2665 nodes (Gene: 2656, miRNA:9) and 3170 edges (Figure 8A), while 109 nodes (Gene: 108, miRNA:1) and 108 edges were associated with the single down-regulated miRNA (**Figure 9B**). Inclusion of both up- and down-regulated miRNAs resulted in a network comprising 2722 nodes (Gene: 2712, miRNA:10) and 3278 edges (**Figure 9C**). In the network generated by the up-regulated DE miRNAs (referred to as network 1), 365 target genes were regulated by 2 miRNAs (degrees=2) while 68 genes had degrees ≥3. Moreover, 8 genes were each targeted by 4 miRNAs (degrees=4), and those genes targeted by the most miRNAs were *NUFIP2* (degrees=6) and *NR2C2* (degrees=5) (**Annexure D: Table 21**). The network comprising the target genes generated by both the up- and down-regulated DE miRNAs (referred to as network 2) demonstrated 395 target genes regulated by 2 miRNAs (degrees=2), whereas 76 genes had degrees ≥3 and 11 were targeted by 4 miRNAs (degrees=4). The genes regulated by the most miRNAs were *NR2C2* (degrees=6), *NUFIP2* (degrees=6) and *VEGFA* (degrees=5) (**Annexure D: Table 22**). All the genes identified with the higher degrees (≥3) from network 2 demonstrated overlap in network 1, while 8 non-overlapping target genes in network 1, had displayed connections with the down-regulated miRNA node (hsa-miR-504-5p). The target genes derived from the single down-regulated miRNAs would only have connections to that respective miRNA and thus have resultant degrees of 1.

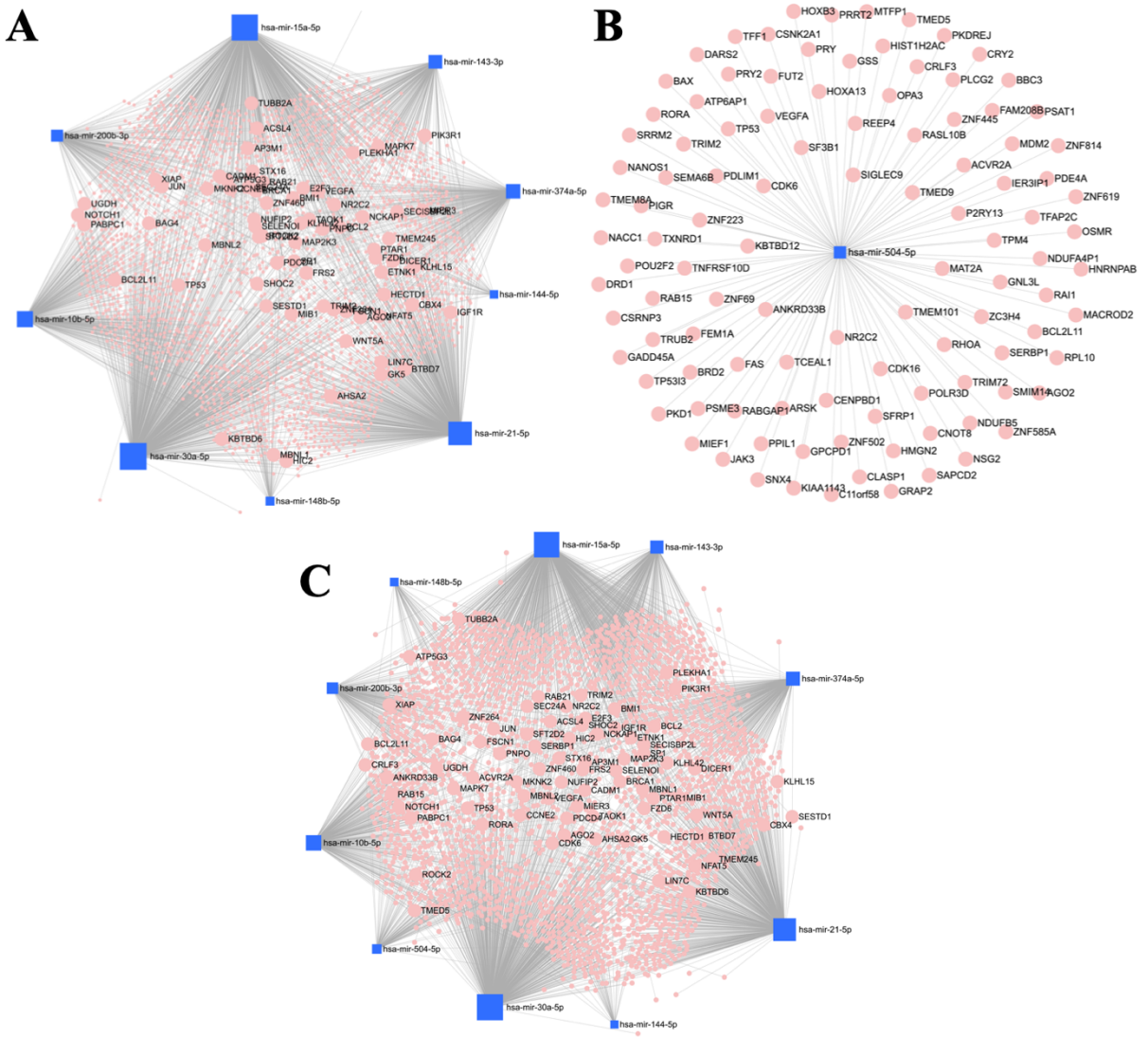
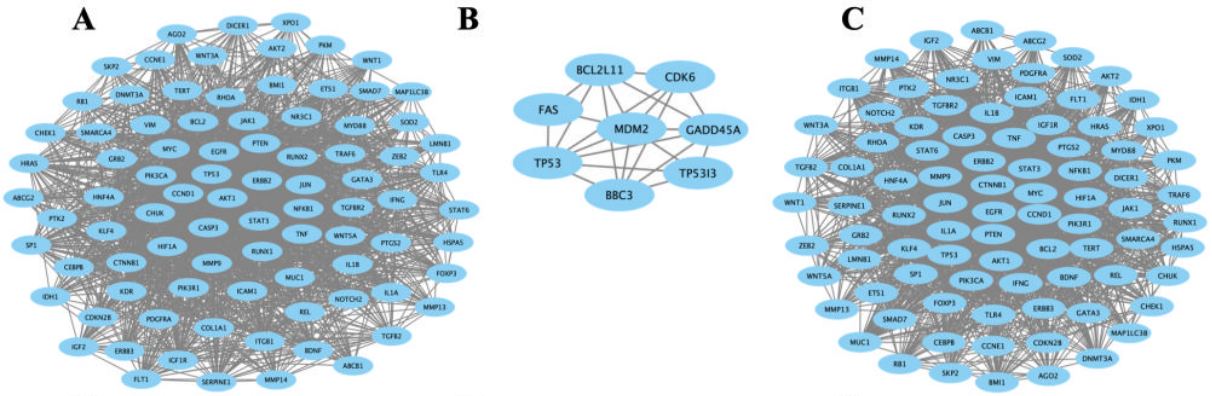


Figure 9: Predicted target genes of DE miRNAs: **A**: Up-regulated DE miRNA-target gene network. **B**: Down-regulated DE miRNA-target gene network. **C**: Up- and down-regulated DE miRNA-target gene network. The squares (nodes) represent the DE miRNAs (node size relates directly to the target gene amounts and significance), the circles represent the target genes, enlarged circles represent target genes considered important due to several miRNA connections; and the grey lines (edges) represent the interactions between miRNA and the target genes (DE – differentially expressed).

11.2.2. Construction of protein-protein interaction networks and top hub genes

Protein-protein interactions (PPI) networks were constructed for the target genes of the up- and down-regulated miRNAs, as well as for their combination. For up-regulated miRNAs, the associated PPI network consisted of 2637 nodes and 46432 edges (**Annexure D: Figure 13**), and the target gene network of the single down-regulated miRNA had 107 nodes and 80 edges (**Annexure D: Figure 14**). The PPI network of the combined network comprising both up- and down-regulated miRNA target genes had 2692 nodes and 47670 edges (**Annexure D: Figure 15** It should be noted that unlike with the up-regulated and combined network, the PPI enrichment value for the network retrieved from the down-regulated miRNA was not significant ($P<0.04$ versus $P=0.0913$). Vital modules (clusters) in the PPI networks were obtained with the MCODE plug-in and showed that the up-regulated network had 87

nodes, 1872 edges and a MCODE score=43.54. Whereas the down-regulated network comprised 8 nodes, 22 edges and a MCODE score=6.29. The module extracted from the combined network had the same characteristics as the up-regulated network (**Figures 10 A-C**). Hub-genes of the PPI networks were identified with the cytoHubba plug-in and the top 10, ranked by degree, were demonstrated for each network. For the up-regulated network, the top 10 hub genes (ranked from highest to lowest) were tumor protein 53 (*TP53*), AKT serine/threonine kinase 1 (*AKT1*), Myc myelocytomatosis oncogene (*MYC*), catenin beta 1 (*CTNNB1*), epidermal growth factor receptor (*EGFR*), phosphatase and tensin homolog (*PTEN*), Jun proto-oncogene, AP-1 transcription factor subunit (*JUN*), signal transducer and activation of transcription 3 (*STAT3*), E1A-binding protein p300 (*EP300*) and tissue necrosis factor (*TNF*). The down-regulated network's top 10 (ranked from highest to lowest) comprised *TP53*, murine double minute-2 (*MDM2)*, B-cell lymphoma-2-like protein 11 (*BCL2L11)*, *AGO2*, cyclin-dependent kinase 6 (*CDK6*), B-cell lymphoma-2 binding component 3 (*BBC3*), growth arrest and DNA damage-inducible protein GADD45 alpha (*GADD45A*), BCL-2 associated X protein (*BAX*), fas cell surface death receptor (*FAS*), and Ras homolog family, member A (*RHOA*) (**Figures 10 D-F**). All the hub genes of the up-regulated network were also observed with the combined (up- and down-regulated) network. The *TP53* hub gene was not only observed in all 3 ranking lists but also had the highest-ranking count for all 3 network (**Figures 10 G-I**).



G

	TP53 (ENSP00000269305)	608.0
	AKT1 (ENSP00000451828)	492.0
	MYC (ENSP00000478887)	488.0
	CTNNB1 (ENSP00000495360)	439.0
	EGFR (ENSP00000275493)	415.0
	PTEN (ENSP00000361021)	345.0
	JUN (ENSP00000360266)	337.0
	STAT3 (ENSP00000264657)	335.0
	EP300 (ENSP00000263253)	329.0
	TNF (ENSP00000398698)	322.0

H

	TP53 (ENSP00000269305)	21.0
	MDM2 (ENSP00000258149)	11.0
	BCL2L11 (ENSP00000376943)	9.0
	AGO2 (ENSP00000220592)	7.0
	CDK6 (ENSP00000265734)	7.0
	BBC3 (ENSP00000404503)	7.0
	GADD45A (ENSP00000360025)	6.0
	BAX (ENSP00000293288)	5.0
	FAS (ENSP00000498466)	5.0
	RHOA (ENSP00000400175)	5.0

I

	TP53 (ENSP00000269305)	616.0
	AKT1 (ENSP00000451828)	495.0
	MYC (ENSP00000478887)	494.0
	CTNNB1 (ENSP00000495360)	443.0
	EGFR (ENSP00000275493)	419.0
	PTEN (ENSP00000361021)	348.0
	JUN (ENSP00000360266)	342.0
	STAT3 (ENSP00000264657)	339.0
	EP300 (ENSP00000263253)	334.0
	TNF (ENSP00000398698)	328.0

Figure 10: PPIs comprising the target genes of the DE miRNAs: **A**: Pivotal module of target genes extracted from the PPI network of up-regulated miRNAs. **B**: Pivotal module of target genes extracted from the PPI network of down-regulated miRNAs. **C**: Pivotal module of target genes extracted from the PPI network of the combined (up- and down-regulated) miRNAs. **D&G**: Top 10 hub genes and respective ranking count identified in the PPI network of up-regulated miRNAs. **E&H**: Top 10 hub genes and respective ranking count identified in the PPI network of down-regulated miRNAs. **F&I**: Top 10 hub genes and respective ranking count identified in the PPI network of the combined miRNAs. The ellipses (nodes) represent the DE genes, the grey lines (edges) represent the interactions, and the colour intensity (D-I) has a direct relationship with degrees of connection (PPI – protein-protein interaction, DE – differentially expressed).

11.2.3. Functional enrichment analysis of the hub genes in the protein-protein interaction networks

Since hundreds to thousands of genes were targets of the DE MiRNAs, to improve specificity, only the top 10 hub genes in the PPI networks were subjected to functional analysis in order to determine which pathways were specifically enriched. Since the pathways identified in the PPI networks of the up-regulated and combined miRNA-mRNA networks were identical, **Figure 11** is therefore a representation of the enriched outputs for both networks. A total of 714 GO BP terms were significantly enriched and the top 10 terms predominantly included transcription-related processes namely positive regulation of miRNA transcription, nucleic acid-templated transcription, miRNA transcription, DNA-template transcription, DNA-binding transcription factor activity, transcription by RNA-polymerase and DNA-templated transcription. Additional BP terms namely positive regulation of miRNA metabolic process, regulation of cell population proliferation and apoptotic process, were also enriched. KEGG analysis revealed 131 significantly enriched pathways, whereas Reactome and PANTHER had 329 and 30, respectively. Nearly all the top KEGG pathways were cancer-related barring two, Hepatitis C and B. The top enriched Reactome pathways included signaling by neurogenic locus notch homolog protein (Notch), signal transduction, IL-4 and -13 signaling, developmental biology, generic and RNA polymerase II transcription pathway, immune system and estrogen receptor (ERs)-mediated signaling pathway. The following was enriched with PANTHER analysis: p53 pathway and p53 pathway feedback loop 2, Huntington disease, Angiogenesis, Rat sarcoma (Ras) pathway and several signaling pathways with particular emphasis on cholecystokinin receptor (CCKR), Wnt, apoptosis, IL, and inflammation mediated by chemokine and cytokine (**Figure 11**).

The top hub genes in the PPI network generated from the single down-regulated miRNA's target genes were significantly enriched in 259 GO BP terms as well as in 63 KEGG, 136 Reactome and 15 PANTHER pathways. GO BP terms were mostly enriched for either *(1)* mitochondrial- (positive regulation of release of Cytochrome C, regulation of release of Cytochrome C, and positive regulation of mitochondrion organization) or *(2)* apoptotic-related processes (apoptotic process, positive regulation of apoptotic signaling pathway, positive regulation of apoptotic process, intrinsic apoptotic signaling pathway and regulation of apoptotic process). Additional BP enriched terms were positive regulation of

programmed cell death and response to endoplasmic reticulum stress. At least half of the enriched KEGG pathways were cancer-related, while the other half consisted of p53 signaling pathway, Epstein-Barr virus infection, apoptosis, human cytomegalovirus infection and measles. Reactome analysis demonstrated 4 TP53-transcription-related pathways (transcriptional regulation by TP53, genes involved in G2 cell cycle arrest, cytochrome c release and cell death genes), and 3 additional transcription-related pathways (generic transcription pathway, RNA polymerase II transcription and gene expression). The remaining top 10 pathways included intrinsic pathway for apoptosis, activation of BH3-only proteins and oncogene-induced senescence. With Reactome, the commonly enriched pathways were affiliated with 4 p53-involved pathways namely p53 pathway, p53 pathway feedback loops 1 and 2, and p53 pathway by glucose deprivation. Apoptosis signaling, FAS signaling and p38 mitogen-activated protein kinase (MAPK) pathways were also enriched. The remaining 3 that make up the top 10 included Huntington disease, CCKR signaling map, and axon guidance mediated by semaphorins (**Figure 12**).

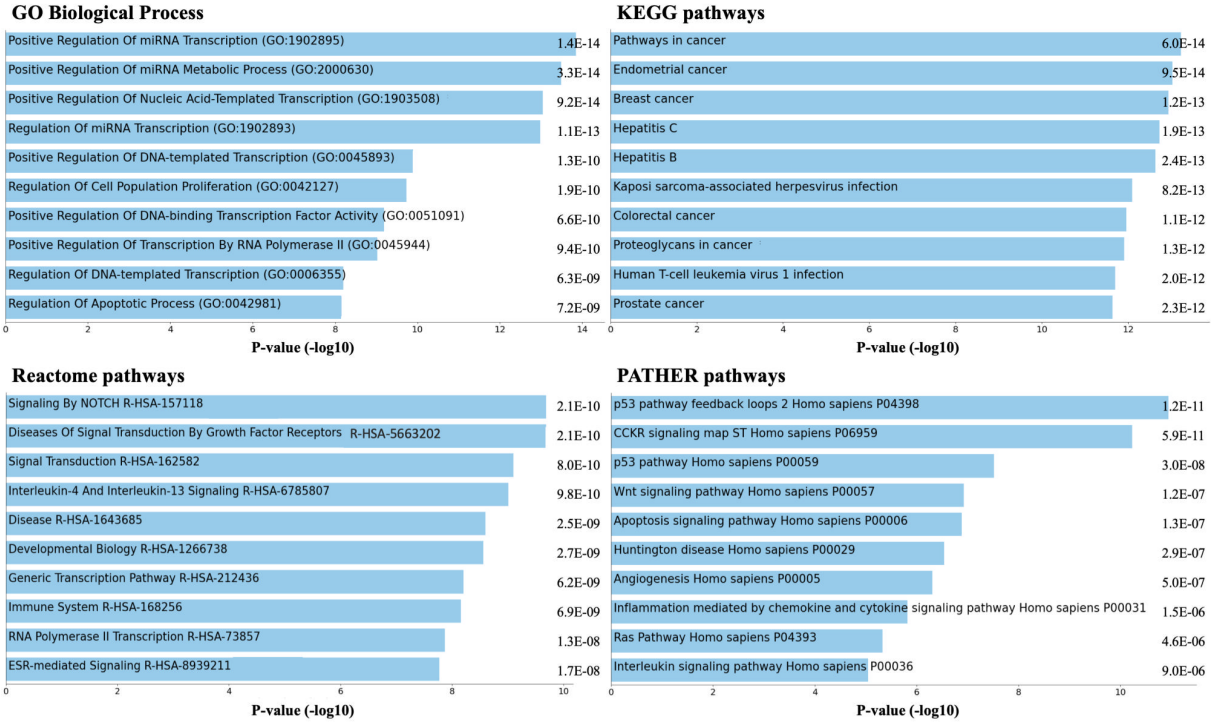


Figure 11: Enriched terms and pathways of the top 10 hub genes from the PPI networks: Top 10 enriched GO BP terms, KEGG, Reactome and PANTHER pathways (Padj.<0.05) of the top 10 hub genes from the PPI networks associated with the up- and the combined (up- and down-regulated) miRNAs-mRNA networks. Bar length corresponds to the adjusted P-value of the enriched term/pathway in descending order (PPI – protein-protein interaction, GO – gene ontology, KEGG - Kyoto Encyclopedia of Genes and Genomes).

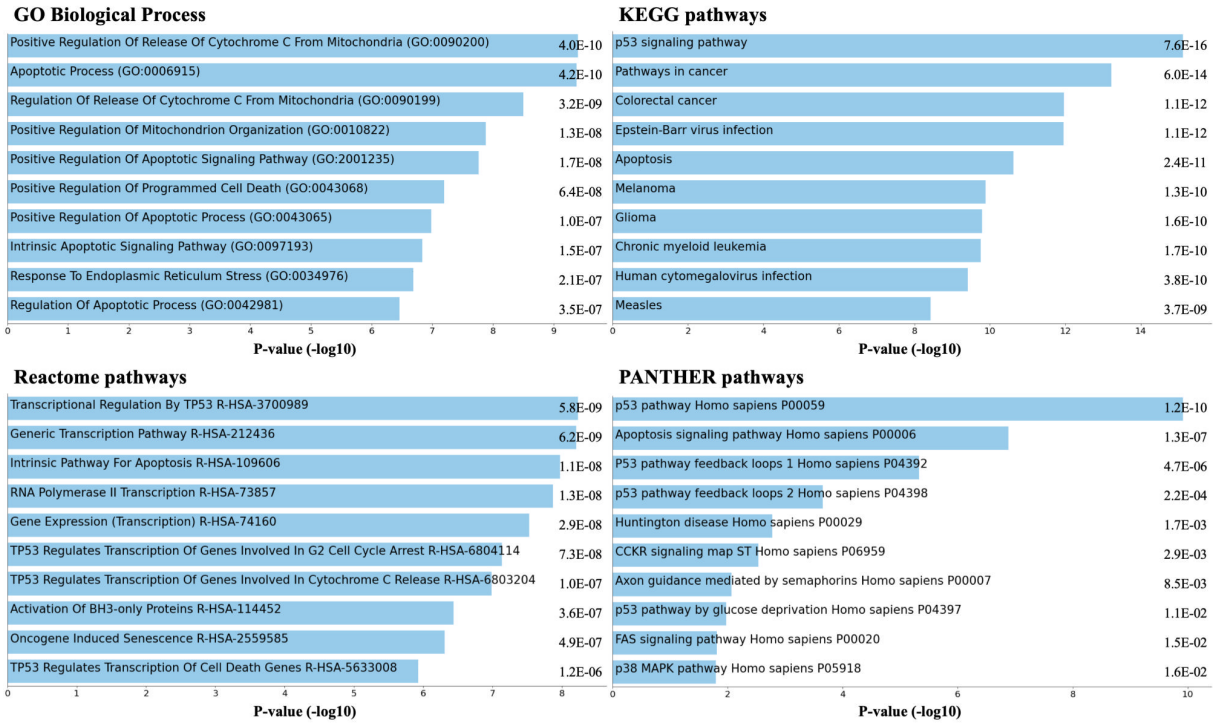


Figure 12: Enriched terms and pathways of the top 10 hub genes from the PPI networks: Top 10 enriched GO BP terms, KEGG, Reactome and PANTHER pathways (Padj.<0.05) of the top 10 hub genes from the PPI networks associated with the down-regulated miRNAs-mRNA network. Bar length corresponds to the adjusted P-value of the enriched term/pathway in descending order (PPI – protein-protein interaction, GO – gene ontology, KEGG - Kyoto Encyclopedia of Genes and Genomes).

11.3. Discussion

11.3.1. Up-regulated differentially expressed miRNAs target genes and pathways for inhalation injury based on their role in inflammation and/or apoptosis

Ten miRNAs, based on differential abundance and fold change significance, were identified as a potential panel representing inhalation injury. One of these was significant for the mild form and was down-regulated, while the remaining 9 miRNAs were significant for severe inhalation injury and all were up-regulated. The function of these miRNAs in the pathogenesis of inhalation injury was supported by their influence on inflammatory and apoptotic responses accompanying related conditions as discussed in Chapter 6. However, the ability of miRNA to regulate disease outcomes is based on its post-transcriptional function of respective target mRNA genes that in turn control cellular and molecular pathways. Since this was the first study that investigated miRNAs in human whole blood of inhalation injury patients, it was important to determine the target genes of these miRNAs and the respective pathways that were regulated in order to identify potential relationships with inhalation injury and its sequelae. Therefore, the up-regulated, down-regulated and miRNAs combination were subjected to target gene and PPI network analysis. This led to the identification of the vital module (cluster) and top 10 hub genes within the network, which were most influential in the pathways that controlled the disease outcomes. These hub genes were subjected to functional enrichment analysis using GO BP, KEGG,

Reactome and PANTHER in order to determine their significant associations with biological pathways. While GO and KEGG were more commonly used in the literature, the inclusion of Reactome and PANTHER provided a broader analytical scope. Since each tool emphasizes different aspects of biological pathways, the convergence of results across all four (particularly the consistent enrichment in inflammatory and/or apoptotic processes) demonstrates the robustness of the findings. Using multiple enrichment platforms therefore reinforced biological relevance of the hub genes and supported their potential role in inhalation injury which likely originates from the dysregulated miRNA expression profiles.

Ten miRNAs, based on differential abundance and fold change significance, were identified as a potential panel representing inhalation injury. One of these was significant for the mild form and was down-regulated, while the remaining 9 miRNAs were significant for severe inhalation injury and all were up-regulated. The function of these miRNAs in the pathogenesis of inhalation injury was supported by their influence on inflammatory and apoptotic responses accompanying related conditions as discussed in Chapter 6. However, the ability of miRNA to regulate disease outcomes is based on its post-transcriptional function of respective target mRNA genes that in turn control cellular and molecular pathways. Since this was the first study that investigated miRNAs in human whole blood of inhalation injury patients, it was important to determine the target genes of these miRNAs and the respective pathways that were regulated in order to identify potential relationships with inhalation injury and its sequelae. Therefore, the up-regulated, down-regulated and miRNAs combination were subjected to target gene and PPI network analysis. This led to the identification of the vital module (cluster) and top 10 hub genes within the network, which were most influential in the pathways that controlled the disease outcomes. The top hub genes (*AKT1*, *TNF*, *EGFR*, *JUN*, *EP300*, *CTNNB1*, *MYC*, *STAT3*, *TP53,* and *PTEN*) for the up-regulated and combined networks were identical, which may demonstrate the impact of the up-regulated miRNAs within the entire network when compared to the down-regulated miRNA. These target genes were commonly the focus of cancer studies, which was not surprising since the majority of reports investigating miRNA potential as biomarkers were also largely cancer-based. A strong association between cancer and inflammation was observed through common pathways (Barabutis et al., 2018), to such an extent that inflammation was reported as a predisposing factor for cancer (Gudkov et al., 2011). This only strengthened the proposed potential ability for miRNAs to prognosticate inhalation injury through target gene and subsequent pathway modulation in inflammation.

Mutations in certain genes can result in inflammation and contributing events such as DNA damage from ROS and RNS. A positive feedback loop typically occurs where cellular responses to the DNA damage exacerbate inflammation (Kay et al., 2019). *TP53* was the highest-ranked gene in all the networks, and, according to literature, mutations relating to the gene and its p53 protein had pro-inflammatory implications. The mutant isoform of the gene promoted chronic inflammation by

mediating and sustaining NF-κB activation, thereby stimulating constant tissue damage and prolonging inflammation (Cooks et al., 2013). Apart from NF-κB pathway involvement, mutated p53 also coordinated additional inflammatory signaling cascades through TNF induction (Cooks & Harris, 2014), which functions pleiotropically, causing inflammatory and immune responses in pathological conditions (Jang et al., 2021; Horiuchi et al., 2010). It is typically inactive in physiological conditions, which prevents excessive inflammation and would only be released when numerous cells such as T-lymphocytes, macrophages, etc. are activated during pathological conditions (Jang et al., 2021; Horiuchi et al., 2010). The infiltration of these cells from the vascular space into the tissues was dependent on changes in permeability, of which an increase of these cells were synonymous with inflammation. Altered permeability has been induced by *AKT1* and has been the primary isoform in ECs, exerting its functions by altering permeability through concomitant regulation of bradykinin, vascular endothelial (VE)-cadherin, histamine, NOS, and leukocyte extravasation (Di Lorenzo et al., 2009). Effects of the latter mediators in diseases were shown to promote the *AKT1*/eNOS/VE-cadherin and phosphoinositide 3-kinase (PI3K)/Akt (also known as protein kinase B) signaling pathway that led to edema and acute inflammation, respectively (Di Lorenzo et al., 2009).

Another pleiotropic gene, *MYC*, was considered a universal transcription amplifier and, along with *AKT,* was also ranked high in this study. In physiological conditions, cellular(c)-*Myc* (the gene involved in numerous cellular processes such as apoptosis, cell cycle, metabolism, etc.) was moderately expressed in somatic cells, but has been significantly induced after exposure to and engagement with antigens, growth factors, binding proteins, and co-stimulation factors (Nie et al., 2012; Levens, 2010; Domínguez-Cáceres et al., 2004; Bowman et al., 2001; Klemsz et al., 1989). Abnormal expression of the gene can occur in several ways through interactions with (*1*) macrophage-colony-stimulating factor (CSF) (Liu et al., 2016; Cheng et al., 1999), (*2*) IL-4 (Martinez et al., 2013; Pello et al., 2012), (3) tissue growth factor (TGF)-*β* (Liu et al., 2015), *as* well as (*4*) IL-2, T-cell antigen receptor, protein-kinase C and intracellular calcium-dependent signaling (Preston et al., 2015). Additional gene interactions with the latter may also indirectly activate additional c-*Myc*. For instance, activated *RAS* and extracellular signal-regulated (*ERK*) genes were reportedly necessary for the indirect expression of c-*Myc* typically induced by macrophage-CSF (Cheng et al., 1999). Regardless of the direct or indirect effects stimulating *MYC* expression, it induces the release of IL-2, IL-4, and TGF-*β*, in addition to numerous other pro-inflammatory mediators and macrophage activation (Bae et al., 2021; Liu et al., 2015; Preston et al., 2015; Pello et al., 2012). Therefore, once *MYC* has been activated, even in minor increments, its expression would not only stimulate acute inflammation, but the functional capacity to do so is sustained through the resultant positive-feedback loop that potentiates chronic inflammation. Several converging pathways reportedly played a role in indirectly activating *MYC* and these included the Ras/Raf/MEK/MAPK pathway (Cheng et al., 1999), while the continuous expression of *MYC* was stimulated by Janus kinase/signal transducers and activators of transcription (JAK/STAT) signaling pathway (Preston et al., 2015). Also linked to c-*Myc* transactivation was the WNT/*β*-catenin signaling

pathway, which was effective through the relationship between WNT signaling and the subsequent prevention of *β*-catenin ubiquitination. This allowed for *β*-catenin entrance and accumulation in the nucleus, which induced the transcription of c-*Myc* (Zhang et al., 2012; He et al., 1998). *β*-catenin proteins are encoded by the hub gene *CTNNB1* gene, where it reportedly formed and maintained adherens junctions. These were known to play a role in cadherin-mediated adhesion (Zhuang et al., 2023; Gao et al., 2018) which has been pivotal in forming and maintaining EC junctions (Tusavitz et al., 2020). Mutated *CTNNB1* directly impacted *β*-catenin synthesis that resulted in the hyperactivation of the WNT/*β*-catenin signaling pathway (Chiurillo, 2015; Anastas & Moon, 2013), in addition to the pathway also being indirectly activated via the Wls gene required for macrophage-induced WNT signaling (Tusavitz et al., 2020). Regardless of the mode of activation it was reported that, aberrant WNT/*β*-catenin signaling adversely affected endothelial function mediated by excessive inflammatory cytokines, monocyte adhesion and permeability, of which all were ablated to normal levels when the e pathway was inhibited (Wadey et al., 2023).

Further to *β*-catenin and WNT signaling, the *EGFR* hub gene also demonstrated its involvement in the pathway. Belonging to a family of receptor tyrosine kinases known to regulate *β*-catenin (Brembeck et al., 2006; Lu et al., 2003), it reportedly caused perturbations in epithelial-cadherin-mediated cell adhesion functions (Lee et al., 2010),affecting epithelial permeability. Moreover, elevated LPS-generated oxidative stress (Gao et al., 2013), inflammatory chemokines, and related cytokines were suggestive of *EGFR* activation in macrophages (Elkamhawy et al., 2019; Hardbower et al., 2016). These observations were supported when *EGFR* inhibitors counteracted LPS-induced iNOS expression, the production of NO, IL-1β, IL-6, TNF-α (Elkamhawy et al., 2019), and oxidative stress (Wang et al., 2017). Similar findings illustrated augmented NO, cytokines, and chemokines in macrophages when *EGFR* signaling was prevented (Hardbower et al., 2016). Its impact on numerous tissues was not surprising since its expression was observed in ECs, vascular smooth muscle cells (SMCs), cardiomyocytes, and macrophages (Schreier et al., 2014). Therefore, any pro-inflammatory mediators released in abundance by activated *EGFR* would potentially have widespread pathological implications. This was further supported through regulatory functions in numerous inflammatory conditions, including viral-induced respiratory inflammation (Xu et al., 2011), thrombin-mediated inflammation in intervertebral discs (Huang et al., 2017), palmitic acid-induced inflammation in cardiac muscle cells (Li et al., 2016), neuroinflammation (Qu et al., 2012), and skin inflammation (Mascia et al., 2003). While EGFR's impact on the WNT/*β*-catenin signaling pathway was a proposed mechanism for the reduced permeability and augmented inflammatory mediators (Wadey et al., 2023), its expression also exhibited its effect by modulating additional pathways. The EGFR/MAPK (Qu et al., 2012; Elkamhawy et al., 2019), EGFR/ERK (ERK is part of the MAPK family) (Mascia et al., 2003; Wetzker & Böhmer, 2003; Pearson et al., 2001), and NF-κB pathways (Li et al., 2021c; De et al., 2014; Yamamoto & Gaynor, 2001) have also been implicated in the subsequent inflammatory outcomes of *EGFR*'s activated form.

Significant cross-regulation was observed between WNT/*β*-catenin signaling and NF-κB pathways through the ability of numerous components to act pleiotropically or synergistically, and ultimately participate in inflammation and related disease (Ma & Hottiger, 2016). The NF-κB protein and its associated pathway were also shown to be regulated, by the activation of the remaining hub genes, i.e. *PTEN* (Oh et al., 2023; Cao et al., 2018), *JUN* (Mishra et al., 2016; Stein et al., 1993), *STAT3* (Ageeva et al., 2024; Fan et al., 2013), *EP300* (Mukherjee et al., 2013; Ravi et al., 1998), and *TNF* (Hayden & Ghosh, 2014; Lee et al., 2014). These genes, in addition to many other stimuli (i.e. TNF-α (Hayden & Ghosh, 2014), IL-1β (Scholz et al., 2013), LPS (Qin et al., 2005), and ROS (Morgan & Liu, 2011)) regulate the translocation of *NF-κB* into the nucleus, resulting in transcription of or interaction with numerous co-activators (Mukherjee et al., 2013) or genes (Guo et al., 2024) that activate chemokines, cytokines and adhesion molecules (Hayden & Ghosh, 2014; Kida et al., 2005; Baeuerle & Henkel, 1994) involved in cellular immune and inflammatory processes as well as stress-responsive gene transcription (Guo et al., 2024; Lee et al., 2014; Mukherjee et al., 2013). Thus, these pathological consequences do not solely result from the signaling of *NF-κB*, but also when *NF-κB* directly or indirectly interacts with other molecules (Liu et al., 2017) to activate additional classical signaling pathways, namely PI3K/AKT, MAPK, JAK/STAT, TGF-β, WNT, and Notch (Guo et al., 2024). This would also apply to any of the top 10 identified genes, as they not only regulated a single pathway, but were also capable of recruiting and transcribing other genes, thereby co-regulating and activating many signaling pathways simultaneously. For instance, reduced *PTEN* elevated *PI3K* expression (Millis et al., 2016; Sahin et al., 2014), which in turn elevated downstream signaling to *AKT* and mammalian target of rapamycin (mTOR) (Millis et al., 2016). Therefore, although *AKT* was directly regulated by PI3K/Akt signaling, *PTEN* indirectly regulated it, along with the mTOR signaling pathway, to ultimately negatively impact immune system functions (Vidotto et al., 2020). Similarly, *NF-κB* can mediate both *AKT* and *PTEN*, demonstrating the complex regulation through the PI3K/AKT/NF-κB pathway (Oh et al., 2023). Besides its respective pathway, *NF-κB* together with *STAT3* can also induce chemokines, cytokines, and genes in hypoxia (Dauer et al., 2005; Grivennikov & Karin, 2010). Moreover, *STAT3* activation can target c-Jun (Hirano et al., 2000), which was also an observed target of *NF-κB* in LPS-induced macrophages. The interaction between the latter duo co-regulated Fos-related antigen(*FRA*)-1, which was a gene involved in pro-inflammatory activation states (Zeng et al., 2022). FRA-1 could also be regulated by *ERK* (Mishra et al., 2016) and, together with p38 MAPK and c-Jun N-terminal kinase, mediated c-Jun and c-Fos (Kida et al., 2005). Since c-Fos and *FRA-1* were both members of the same (Fos) family of AP-1 transcription factors (Milde-Langosch et al., 2004), the involvement of c-Fos in inflammation is highly possible.

In hindsight, target genes would naturally regulate their respective canonical pathways (e.g. *TP53* would regulate the p53 pathway, *NOTCH* would regulate the NOTCH pathway, etc.); therefore, these pathways would automatically be enriched. However, the activated target genes may converge and function in other pathways as well. In order to determine the biological significance of the hub target genes relative

to the up-regulated DE miRNAs in this study, functional enrichment analysis was conducted to demonstrate their potential implications in pathways. Most of the GO BP terms demonstrated enrichment in transcription-related pathways, which alluded to the role of miRNAs to inhibit or activate transcription of target genes and subsequent expression that in turn triggers transcription of other genes (Catalanotto et al., 2016). The 'apoptotic process and regulation of cell population proliferation' GO BP terms were also enriched. During nearly any injury, inflammation had triggered differential cell proliferation for wound closure, a mechanism that ceases once the stimuli is removed or the tissue is repaired. However, activated cells or those with DNA damage continued to proliferate under the influence of inflammatory mediators (Coussens & Werb, 2002). This concept had been particularly important in tumorigenesis, but its relevance to inhalation injury lie in the excessive proliferation of activated cells to continuously release inflammatory mediators and trigger classical signaling pathways, thereby exacerbating the outcomes of the initial insult and also promoted chronic inflammation. Typically, this type of abnormal cell proliferation had been physiologically controlled by apoptosis (Li et al., 2004), but in patients with ARDS or ALI, apoptosis resulted in damaged lung epithelial cells (Albertine et al., 2002; Ware & Matthay, 2001; Matute-Bello et al., 1999; Song et al., 1999). The presence of apoptotic pathways was suggestive of LPS-induced inflammation (Kawasaki et al., 2000), while resultant endothelial cell apoptosis was also directly indicative of TNF-α presence (Guinee et al., 1996). Furthermore, the outcomes of elevated apoptotic activity that resulted in the damaged lung epithelium may have direct consequences on effective gaseous exchange. Given that these (ALI, ARDS, impaired lung function, etc.) were also outcomes of inhalation injury, apoptosis may play as much a part in its sequelae as inflammation does. Moreover, because apoptosis and inflammation were strongly linked to cancer pathogenesis (including excessive cell proliferation and tumorigenesis) due to overlapping mediators and mechanisms, the target genes were predominantly and foreseeably enriched in cancer types following KEGG analysis.

Reactome and PANTHER analysis demonstrated enrichment in several signaling pathways, and all have been involved in eliciting immune and inflammatory responses via the signaling from *NOTCH* (Castro et al., 2021; Christopoulos et al., 2021), estrogen receptor-mediated (Härkönen & Väänänen, 2006; Soucy et al., 2005), IL-4 and -13 (Iwaszko et al., 2021; Junttila, 2018), *RAS* (Sadeghi et al., 2023; Reedquist, 2012), CCKR (Saia et al., 2020; Kern-Lunbery et al., 2024), and WNT (Wadey et al., 2023; Lee et al., 2010). Additionally, since *TP53* was the highest-ranked hub gene across all PPI networks, pathways specifically associated with its regulation were to be expected. PANTHER showed that the most enriched pathways were the p53 pathway and p53 pathway feedback loop 2. The p53 pathway was reportedly activated by DNA-related stress and damage resulting from free radical stress (Harris & Levine, 2005; Oren, 2003; Giaccia & Kastan, 1998). Its protein also interacted with numerous inflammatory agents such as ROS, RNS, cytokines, and *NF-κB*, all known to also cause DNA damage. Moreover, in this study, findings showed that the p53 circuit interacted with most of the hub genes, or their proteins, i.e. *AKT* (Harris & Levine, 2005), *MYC* (Rochlitz et al., 1995), *CTNNB1* (Xiao et al.,

2022), *EGFR* (Ding et al., 2022), *PTEN* (Trotman & Pandolfi, 2003), *JUN* (Gowda et al., 2012), *STAT3* (Pham et al., 2020), *EP300* (Grossman, 2001), and *TNF* (Di Minin et al., 2014). Although p53 pathways were highlighted as the most significantly enriched PANTHER pathway, it was a formality that the associated feedback loop would follow since the initial mediators activating the pathway were also released by this very same pathway. Therefore, this profound transcriptional capacity of p53 may also have been involved in the listed pathways that followed in order of decreasing significance. It would not only have elicited a cascade of inflammatory and immune events by interacting with the aforementioned genes, but its concurrent modifications and interactions with genes/proteins were shown to determine its anti- or pro-apoptotic purpose (Oren, 2003). Thus, enrichment in the apoptosis signaling pathway observed with PANTHER would be highly plausible, further supported by its observed enrichment in GO. The bioinformatics analysis demonstrated that the pathways regulated by these target genes of the up-regulated miRNAs were mostly related to inflammation and apoptosis, both of which were highlighted in the pathological sequelae of inhalation injury, particularly in ARDS (Galani et al., 2010; Bhatia & Moochhala, 2004), ALI (Chopra et al., 2009; Drakopanagiotakis et al., 2008), sepsis (Hotchkiss & Nicholson, 2006; Joshi et al., 2003), and pneumonia (Bordon et al., 2013; Guery et al., 2002). Zhang et al, (2018) corroborated some of these results by demonstrating inflammatory and apoptotic-enriched pathways relative to the DE miRNAs observed in burn-induced lung injury of mouse models (Zhang et al., 2018). The authors concluded that the injury was most likely due to the involvement of the miRNAs in the resultant inflammation and apoptosis (Zhang et al., 2018). In another study, when miRNA expression and subsequent pathway enrichment were assessed in deep partial thickness burn autografts, numerous inflammatory and apoptotic signaling pathways were enriched with KEGG. Some of these pathways (JAK/STAT and MAPK signaling pathways) also overlapped with the current KEGG results. The findings in this sub-section demonstrated the potential direct or indirect implications of the up-regulated DE miRNAs target genes in pathways that subsequently modulated the apoptotic and inflammatory events relative to severe inhalation injury and its complications.

11.3.2. Down-regulated differentially expressed miRNAs target genes and pathways for inhalation injury based on their role in inflammation and/or apoptosis

Only one miRNA (hsa-miR-504-5p) was differentially expressed and was also down-regulated in the samples representing mild inhalation injury. This miRNA was one of three of the lower-ranked miRNAs according to degree of connectivity, and when the PPIs were retrieved by STRING, the network was returned as not significant. By STRING definition, the insignificant value suggested that the proteins were not very well connected (the nodes might be random and edges insignificant), but the potential of the proteins to be biologically meaningful should not be excluded, or that the proteins and interactions may not have been extensively studied or known to STRING, respectively. Moreover, individual miRNAs were still able to function alone in gene targeting (Stark et al., 2021), despite the functional efficiency of miRNA clusters (Niu et al., 2023). With this under consideration, the biological potential of the respective target genes and possible links to inhalation injury were still discussed. *TP53* was also

the highest-ranked gene, as observed with the up-regulated and combined miRNA-mRNA networks. Since its ability as a potent mediator of chronic inflammation (Jang et al., 2021; Cooks et al., 2013; Horiuchi et al., 2010) was already described in the previous sub-section (See 11.1.1.), emphasis was subsequently placed on the remaining hub target genes: *MDM2*, *BCL2L11*, *AGO2*, *CDK6*, *BBC3*, *GADD45A*, *BAX*, *FAS,* and *RHOA*. The *MDM2* gene elicits glycolytic and pro-inflammatory responses by increasing IL-1β and NO in macrophages (Wu et al., 2024). The application of an *MDM2* blockade reduced pro-inflammatory cytokines, chemokines, and leukocyte recruitment (Mulay et al., 2012), as well as suppressed LPS-induced lung inflammation seen in both ALI (Liu et al., 2009b) and vascular SMC inflammation (Hashimoto et al., 2011; Ihling et al., 1998). In addition, *MDM2* also controlled apoptosis by interacting with *NF-κB* and even regulating the expression of p53 (Mulay et al., 2012; Thomasova et al., 2012). Therefore, p53 not only had inflammatory capabilities, but it also had clear implications in the mechanisms of apoptosis (Barabutis, 2020; Speidel, 2010).

Apoptosis can be divided into extrinsic or intrinsic apoptotic pathways when triggered by external or internal stimuli, respectively. The mechanisms driving these pathways also differ and were mitochondrial-dependent for the intrinsic pathway, while the extrinsic was death receptor-mediated (Singh et al., 2019; Luo & Rubinsztein, 2013). The intrinsic apoptotic pathway has largely been controlled by the BCL2-family, which included the *BCL2L11* (or BIM) and *BBC3* (or p53 upregulated modulator of apoptosis-PUMA). These were BH3-only protein subclasses with the former being the first identified molecule having both anti-autophagy and pro-apoptotic properties (Luo & Rubinsztein, 2013), and the latter reportedly being one of the most potent apoptotic proteins (Omori et al., 2011; Vousden, 2005). Pro-apoptotic activation (e.g. cellular stress or impaired signals) (Luo & Rubinsztein, 2013; Rathmell & Thompson, 2002) resulted in the activation of BH3-only proteins, which binds to and inhibits other BCL-2 proteins (Luo & Rubinsztein, 2013; Lamhamedi-Cherradi et al., 2003). These essentially protect the integrity of the mitochondria's outer membrane by inhibiting *BAX* (Bock & Tait, 2020). In the absence of BCL-2, *BAX* would translocate from the cytosol into the mitochondria and make the outer membrane porous. This promoted membrane disruption, allowing for efflux of several proteins (such as cytochrome c) into the cytoplasm (Gurzov et al., 2010; Gross et al., 1998; Kluck et al., 1997) that in turn activated caspases and resulted in apoptosis (Gurzov et al., 2010; Gross et al., 1998; Li et al., 1997). *BBC3* followed this same mechanism after activation by *NF-κB* (Gurzov et al., 2010; Gross et al., 1998), but could also act directly through p53-related components (Yu et al., 2003) and indirectly through other transcription mediators (You et al., 2006; Reimertz et al., 2003). Moreover, expression of the hub gene, stress protein *GADD45A*, also impacted BIM functions. Stress signals or DNA damage induced the expression of *GADD45A,* which caused BIM translocation and accumulation in the mitochondria. The interactions of BIM with other BCL-2 proteins allowed for the detachment of *BAX* from BCL-2 protein-bound complexes that ultimately would cause cytoskeleton instability, membrane disruption, and apoptosis (Tong et al., 2005).

BBC3, *BCL2L11*, *BAX,* and *GADD45A* were hub genes in this study that were all able to regulate the intrinsic apoptotic pathway (Omori et al., 2011), but the extrinsic apoptotic pathway involving death receptors could be linked to another hub gene *viz. FAS*. Upon binding to its receptor, *FAS* would subsequently form the death-inducing signaling complex comprising itself, a procaspase, and an associated death domain protein (Luo et al., 1998). In response to this complex, a series of caspases were activated, and DNA fragmentation and apoptosis were induced (Bock & Tait, 2020; Luo et al., 1998). It was also reported that, as a consequence of interaction with death stimuli, *BCL2L11* was phosphorylated via *FAS* and subsequently resulted in apoptosis (Luo & Rubinsztein, 2013). Therefore, *FAS* was able to converge and engage with the intrinsic pathway through BH3-only/BCL-2 family member cleavage that resulted in exacerbated caspase activation and amplified apoptosis (Luo et al., 1998). Apoptosis was an important pathological consequence in burns (Kubo et al., 2014; Lootens et al., 2013) which, along with inflammatory outcomes were investigated in serum retrieved from rat models with varying degrees of scald burns (Wang et al., 2018). Wang and colleagues (2018) particularly focused on the pro-apoptotic *BAX* and anti-apoptotic *BCL-2* mRNA and proteins, and the pro-inflammatory mediator, TNF-α. The authors observed reduced *BCL-2* and increased *BAX* and TNF-α correlated with burn degree and with time. They speculated that with increased degree, a stronger inflammatory response was potentially related to excessive and continuous TNF-α release, whereas increased degree also directly correlated with tissue damage relative to excessive and continuous *BAX* stimulation. Several studies have reported on the close management of apoptosis with *BAX* and *BCL-2*, and the inflammatory implications of TNF-α (Jiang et al., 2014; Hoshyar et al., 2013). The degree and development of burn injuries were demonstrably linked to factors of both apoptosis and inflammation; therefore, these factors may be related to clinical outcomes of inhalation injury. A relationship also involving inflammation and apoptosis was observed with *GADD45A* expression, which subsequently activated p53 through the modulation of the p38 MAPK signaling pathway (Salvador et al., 2013). Another mechanism of *GADD45A* was its ability to activate the AKT-mediated endothelial signaling implicated in inflammatory lung injury and vascular barrier expression (Meyer et al., 2009).

Co-activation and/or dual functioning of target genes was reported as a recurring theme in disease pathology, and hub genes, *CDK6* and *RHOA*, also followed this trend. CDKs typically regulated cell cycle events; however, growing evidence supported their regulatory capacity in inflammatory responses and cell death, independent of cell cycle consequences (Schmitz & Kracht, 2016). Wu et al, (2021) reported that LPS-induced damage in kidney cells resulted in the elevated mRNA and protein components of IL-1β, IL-6, IL-8, and TNF-α, alongside augmented CDK6-AS1 (which is an amplifier RNA that positively correlated with CDK6 (Yang et al., 2022)). When CDK6-AS1 was silenced, the LPS-induced release of pro-inflammatory components, cell apoptosis, and reduced mitochondrial membrane potential were augmented. This study also demonstrated a link between *CDK6* and *BCL-2* by reporting that LPS insult reduced BCL-2 levels, increased *BAX*, and cleaved several caspases; outcomes that were alleviated by CDK6-AS1 silencing (Wu et al., 2021). Moreover, *CDK6* expression

have frequently been involved in the coactivation of *NF-κB*, IL-8, *STAT*, and *AP-1*, thereby demonstrating its potential in pathological conditions where inflammation and apoptosis were ratified (Schmitz & Kracht, 2016; Handschick et al., 2014). Contrary to this, despite *RHOA* being ranked the lowest hub gene in this study, a growing number of studies have demonstrated its importance in inflammation through the interaction with several proteins. Encoded by the gene *RHOA,* RhoA is a small GTPase protein in the Rho family that undergoes downstream effects by Rho-associated protein kinase (ROCK) (Deng et al., 2019). RhoA and ROCK were regulated by p53 through Notch1 (Lefort et al., 2007), an outcome that was supported by the suppression of p53 resulting in the activation of these proteins (Xia & Land, 2007). Together, this Rho/ROCK signaling pathway affected several cellular mechanisms, such as development and migration (Chen et al., 2013b; Loirand et al., 2006) and, as a result, was closely related to inflammation (Xu & Lin, 2024; Zhang et al., 2020b) and epithelial barrier disruption (Xu & Lin, 2024; Akhter et al., 2020; Barabutis, 2020). Its inflammatory role was also suspected as a potential contributor to ALI and ARDS (Fiala et al., 2020; Barabutis, 2019; Coudroy et al., 2019). Moreover, targeting the RhoA/ROCK pathway was suggested to improve inflammatory pain induced by LPS since its signaling elevated pro-nociceptive cytokines (i.e. TNF-α and IL-1β), which typically caused hyperalgesia (Wang et al., 2015). LPS treatment also activated and translocated RhoA, but was reportedly dependent on NF-κB, especially since its respective depletion inhibited the LPS-induced translocation of RhoA (Tao et al., 2012). LPS not only induced major inflammation-causing pro-inflammatory toxins and cytokines, but after it activated and translocated RhoA, RhoA in turn enhanced LPS-induced IL-6 and -8 secretion (Tao et al., 2012) and also indirectly activated NF-κB (Tong & Tergaonkar, 2014). Interestingly, both RhoA and NF-κB depletion resulted in a similar reduction of LPS-induced pro-inflammatory effects (Tao et al., 2012),demonstrating their independent impact on inflammation, but also their potential to synergistically exacerbate it. While data on the pathophysiological connection between RhoA and inflammation was more prevalent, its participation in apoptosis was less investigated. Depending on the cell type, RhoA displayed both pro- and anti-survival functions relative to apoptosis occurrence. For instance, RhoA favourably induced apoptosis in cancer-related cells (De Sarno et al., 2005); however, overexpression also resulted in apoptosis in normal functioning cells (Esteve et al., 1998). Unsurprisingly, its apoptotic role in cardiomyocytes was related to *BAX* as well (Del Re et al., 2007), thus reinforcing the primary causative apoptotic function of the BLC-2 family of proteins.

Arguably, of all target genes that were regulated by the DE down-regulated miRNA (hsa-miR-504-5p) in this study, *AGO2* was the most unsurprising observation. *AGO2* was described as being a key component of miRNA biogenesis and as the catalytic component in RISC, *AGO2* was bound to miRNA and not only facilitated miRNA generation, but also acted as a guide to the complementary mRNA target gene for subsequent silencing or interference (Meister, 2013; Hutvagner & Simard, 2008). Since *AGO2* plays a large functional role relative to miRNA, it has not typically been considered a target gene thereof. However, the observation that *AGO2* was indeed a hub target gene in this study can be explained by the

potential of the other target genes to activate its expression. While very little information on these *AGO2*-activating genes exists, one study demonstrated the ability of the p300 protein (encoded by *EP300*) to impact *AGO2*, resulting in the recruitment and maturation of the attached miRNA. Although beyond the scope of this study, further investigation into *AGO2* would be beneficial in delineating the converging mechanisms involving miRNA target genes and *AGO2*. Since *AGO2* is technically, partially responsible for post-transcriptional functioning, it could be related to some of the pathways that were typically associated with miRNA function in general and were enriched with Reactome, such as 'generic transcription or RNA polymerase II transcription'. Moreover, with the exemption of the predominant enrichment of cancers with KEGG; GO, BP, Reactome, and PANTHER demonstrated functional enrichment primarily involving pathways relating to apoptosis and p53 regulation. This may directly or indirectly correlate with three observations: *(1) TP53* being the highest-ranked hub gene in this study, which was strongly involved in regulating both inflammatory and apoptotic mechanisms; *(2)* the remaining hub target genes of the down-regulated miRNA playing a more dominant role in inducing apoptosis in various cell types; and/or *(3)* the potential of all the target genes to activate apoptosis potentially as an early response to mild inhalation injury and thus possibly contribute to inflammation development or progression. Based on the target genes and enriched pathways, the role of the hsa-miR-504-5p may be more prominent in apoptosis compared to the up-regulated miRNAs, which had stronger inflammatory connections. Despite being less connected to target genes compared to the miRNAs with higher degrees, the few apoptotic-related enriched pathways of the combined miRNAs target genes (the mRNAs of both up- and the down-regulated miRNA) may have been influenced by the down-regulated miRNA that activated target genes mostly involved in apoptosis. However, the target genes and enrichment outputs of the up-regulated miRNA were potentially more potent because they were identical to those of the combined network's outputs even when the down-regulated outputs were considered. Since patients may present with either mild or severe inhalation injury on admission or the condition may worsen over time, determining and mapping the PPIs and enriched pathways of all the miRNAs combined, as well as that of only the up- or down-regulated miRNAs, could provide information on the evolution of the disease from between mild or moderate and severe presentation. Future studies may therefore benefit even more by applying this analysis to samples collected at different clinical time frames to not only potentially represent the evolution of inhalation injury, but also to identify changes in miRNA expression and relate those changes to the pathways affected and the subsequent clinical outcomes presented.

Since the resultant PPIs, modules, hub genes, and enriched pathways were involved in the hallmarks of inhalation injury, i.e. apoptosis and inflammation, this chapter provided the potential foundation supporting the use of the 10 DE miRNAs (see Chapter 6) as a panel for inhalation injury prognostication. Moreover, the hub genes and pathway outcomes of the up-regulated miRNA network were distinctly different from those of the down-regulated network, and since each represented an injury degree, the DE miRNAs also showed potential in identifying the mild injury form from the severe. It should also

be noted that the miRNAs in this study may have functioned more effectively as a network (as seen by the insignificant PPI network for the single miRNA); therefore, the severe degree was better represented compared to the mild form. However, the latter should be considered with caution due to the limitations (discussed later) that could influence the results of the study and alter the panel to include additional miRNAs that could either improve prognostication or provide an additional injury mid-point, such as moderate or moderately severe injury. The latter would aid in creating a grading system indicating varying inhalation injury degrees and correlate with the required treatment. In these cases, if mild injury can be predetermined, then ETI would not be required, or treatment could be less invasive and rely more on bronchodilator or oxygen management. Alternatively, earlier extubating could also occur, which is pivotal as intubation was typically accompanied by its own set of complications (Romanowski et al., 2016), increasing risk to the patient over the intended benefit. Although additional studies would be required to conclude that the miRNAs were definitively correlated to early inhalation injury stages, these preliminary findings were very promising and provide an ideal foundation for future studies. Moreover, the limitations and future perspectives could be considered and applied in order to bridge the gaps between the miRNAs, their implicated genes, and respective pathways with the complex nature of inhalation injury in burn patients.

CHAPTER EIGHT
CONCLUSIONS, LIMITATIONS AND FUTURE PERSPECTIVES

12. Conclusions

Burn patients admitted to the WCPATBC at Tygerberg Hospital, South Africa, predominantly belong to socioeconomically vulnerable populations. As one of only two provincial tertiary units in South Africa specializing in adult burns, it was expected that most cases would involve severe injuries. Given the limited epidemiological data on South African burn centres, the first part of this study aimed to expand the current knowledge base. Findings were consistent with reports from similar clinical settings in South Africa and other LMICs. Key risk factors such as age, male gender, and the predominance of domestic fire, were strongly associated with elevated mortality, thereby highlighting the persistent burden of burn injuries. Continued surveillance of these patterns is critical to improve prevention and treatment strategies in resource-limited settings. Although certain injury-related factors varied from previous reports, such variation was to be expected across cohorts. However, prognostic and diagnostic tools must be sufficiently sensitive to assess the evolving severity and sequelae of burns to reduce mortality risk. While public hospitals deliver quality burn care through innovative strategies and adherence to core burn management principles, the absence of advanced prognostic tools prompts clinicians to rely on the presence of specific subjective/objective indicators and comorbidities for mortality risk assessment.

The well-known mortality co-factors in burn patients have included TBSA, age and gender. Notably, inhalation injury which is a major contributor to poor outcomes, can be present even without typical clinical indicators thereby increasing the risk of misdiagnosis, suboptimal treatment, and adverse outcomes. To clarify its impact in this cohort, the subsequent section examined the relationship between inhalation injury and mortality. Results confirmed its strong correlation and predictive value, consistent with prior literature, and emphasized the need for further investigation into its potential prognosis. Additionally, the study also identified TBSA >40%, the presence of complications (such as ARDS and pneumonia), longer BICU LOS and referrals outside the Tygerberg district as key mortality determinants. These findings may broaden the scope of parameters considered for prognostic assessment, especially the clinical complications generally overlooked in standard evaluations. The risk of burns-related mortality may be reduced by minimizing these key factors, i.e. inhalation injury, however, their prognostic utility is hindered by a lack of consensus definitions and diagnostic criteria for inhalation injury, as well as the limited sensitivity and specificity of existing tools.

Identifying burn-related clinical markers typically recorded at or shortly after admission, could therefore support the early prognosticating of inhalation injury. Four candidate markers were identified: %TBSA, complications, BICU LOS, and lactate levels, with complications and BICU LOS showing the strongest associations. Among these, complications and lactate were suggested as the most suitable parameters

for prognostication, though clinicians would benefit from considering all four candidate factors to monitor disease progression. The observed associations suggest that the increased presence or severity of inhalation injury should be considered when these parameters are elevated. However, given the interconnection of these factors, a multifactorial approach that accounts for both established and emerging prognostic parameters would be essential for improving the assessment of injury onset, progression and subsequent mortality risk. Prognostication should not rely on a single parameter from medical records due to the variable presentation of inhalation injury, disease progression, and coexisting comorbidities. Perhaps sensitive prognostication frameworks may in fact depend on a minimum number of integrated clinical or molecular candidates; although this is a plausible notion, it remains to be systematically explored. Despite the need for further validation, the preliminary basis for identifying such candidates has been demonstrated in this study.

Given these challenges, clinical methods may benefit from the integration of easily attainable, disease-specific biomarkers to potentially enhance prognostic potential. MiRNAs meet this criterion, leading to the final part of this study that investigated differential expression in whole blood from burn patients. Ten differentially expressed miRNAs were identified: One down-regulated (miR-504-5p) in mild injury, and nine up-regulated (miR-143-3p, -200b-3p, -148b-5p, -10b-5p, -30a-5p, -15a-5p, -374a-5p, -21-5p, and -144-5p) in severe injury. These results, supported by two bioinformatics tools and threshold filtering, demonstrated alignment with literature that linked the panel of miRNAs to conditions similar to inhalation injury or its sequelae, and played key roles in apoptosis and inflammation which are hallmarks of inhalation injury. Their biological relevance was further supported by hub gene analysis. The top hub genes from the up-regulated and combined networks were identical and included *AKT1*, *TNF*, *EGFR*, *JUN*, *EP300*, *CTNNB1*, *MYC*, *STAT3*, *TP53,* and *PTEN*. In contrast, the down-regulated network featured *TP53*, *MDM2*, *BCL2L11*, *AGO2*, *CDK6*, *BBC3*, *GADD45A*, *BAX*, *FAS,* and *RHOA*. The association of these genes with inflammation and apoptosis were also previously reported, reinforcing the pathogenic relevance of the miRNA profiles. Specifically, the hub genes of the up-regulated and combined miRNA networks were predominantly involved in inflammatory pathways, whereas those of the down-regulated network were primarily associated with apoptotic responses. Although these associations were distinct, they were not mutually exclusive. The pleiotropic nature of the genes allows for the recruiting and transcribing of other genes, and therefore, would not only directly regulate respective canonical pathways but also indirectly co-regulate and activate multiple downstream signaling pathways simultaneously. Consistent with previous reports, it is likely that the miRNAs functioned more effectively as a cluster than individually. This was supported by the observation that only the up-regulated and combined networks yielded significant PPI networks, whereas the down-regulated network produced fewer connections and shared only one overlapping hub gene. Nonetheless, the latter's relevance should not be understated; as the consistent enrichment of its hub target genes in apoptosis-related pathways suggests a meaningful contribution to the pathophysiology of inhalation injury and its subsequent sequelae.

These trends were reflected in the enrichment analysis, which assessed the biological relevance of hub genes via their pathway involvement. To ensure a comprehensive analytical perspective, the study employed both commonly used tools (GO BP and KEGG) and two additional platforms often cited in literature, *viz.* PANTHER and Reactome. Key pathways related to inflammation and apoptosis were significantly enriched across all platforms. This consistency may be attributed, in part, to the presence of the top-ranking hub gene, *TP53*, across all networks, given its central role in mediating chronic inflammation and its dual function in apoptosis. While apoptotic enrichment was most prominent among the down-regulated miRNA hub genes, inflammatory and immune-related pathways were more strongly represented in the up-regulated and combined networks. Although the down-regulated miRNA appeared to drive some apoptotic activity within the combined network, the up-regulated miRNAs likely had a greater overall influence, as their target genes and enrichment profiles closely mirrored those of the combined analysis. Overall, the distinct differential expression patterns observed, highlight the potential of circulating miRNA profiles to not only indicate the presence of inhalation injury but also reflect its severity. These findings provide a promising foundation for the development of a prognostic or diagnostic miRNA panel, which can only fully be realised when the study's limitations and future research directions are addressed.

13. Limitations

While this study identified potential burn-related clinical markers and biomarkers indicative of the presence and severity of inhalation injury, several limitations should be considered when interpreting the results: (i) The single-time-point measurements from a relatively small sample size at a single-centre population may restrict generalizability of the findings. However, the sample size reflects the total number of eligible patients admitted during the approved study period at the country's only specialized adult burns centre. Patient recruitment was constrained not by study design or logistical oversight, but by the centre's finite infrastructure, strict admission criteria for severe burns, and the variable (often prolonged) LOS associated with injury severity. (ii) Not all medical file data were available prior to analysis. While this had minimal impact on descriptive statistics, it reduced the number of complete cases available for statistical tests requiring pairwise data, such as with traditional correlation and regression analyses. Therefore, incomplete patient files were excluded to preserve statistical integrity, which ensured methodological robustness by restricting analyses to complete cases. (iii) The quality and quantity of available data may have been influenced by the systematic sorting process by medical personnel who were ethically permitted to extract patient information directly from the internal database. While this ensured compliance with data governance protocols, manual data retrieval introduces the potential for human error, omissions, or inconsistencies that may have contributed to missing or incomplete records. These potential limitations emphasizes the need for a standardized and streamlined data-capturing system that could enhance both clinical care and research, particularly in high-acuity burn cases. However, in emergency-driven environments, it would be challenging for clinicians and nurses to collate data for research purposes systematically. A feasible solution may lie in developing

data capture frameworks that closely mirror routine clinical documentation practices, thereby minimizing additional burden while improving data completeness and utility. (iv) Although sample size limitations are unlikely to affect RNA sequencing performance, expanding the dataset may have allowed for a more refined selection of exemplar samples and potentially reveal additional differentially expressed miRNAs. Moreover, a larger cohort could facilitate the inclusion of a control (burn patients without inhalation injury) group to better compare expression profiles across varying injury severities. However, this would require the use of sensitive diagnostic tools capable of confirming the presence or absence of inhalation injury which is currently not accessible in LMIC facilities. Considering all of the above, comparing severe to mild cases remains valuable as it provides insight into gradations of injury severity and their associated molecular responses which is still critical for early prognostication and targeted intervention. (v) While sampling occurred at a single time point on or shortly after admission supporting early prognostication, longitudinal sampling would have enabled stronger validation of the "cause-and-effect" relationships between specific clinical parameters. Thus, distinguishing whether these changes reflect the pathological signals in response to the injury or whether the outcomes were innately compensatory. Moreover, since miRNA expression evolves over time, a time-series approach would enhance the stratification of severity and the identification of temporally relevant biomarkers. Therefore, serial measurements would improve prognostic accuracy by capturing disease progression and the corresponding biomarker dynamics.

14. Future perspectives

This study provides a strong foundation for the potential use of clinical and biological indicators in the prognosis and diagnosis of burns-related inhalation injury and its severity, despite the inherent limitations. Several avenues remain open for further investigation to expand on these findings to validate reliable markers conclusively. Studies suggest that a single clinical marker may not sufficiently predict mortality risk, emphasizing the need to consider multiple markers collectively. Developing a prognostic model incorporating these markers could enhance predictive accuracy. However, additional analyses, such as receiver operating characteristic curves, are necessary to assess the sensitivity and specificity of the marker before model development. This could be complemented by survival analyses, such as Kaplan-Meier or Cox regression, which, while primarily used for mortality prediction, could indirectly assess inhalation injury given its established role as a mortality co-factor. Additional validation studies are essential to confirm the preliminary findings of this study and to establish a more reliable panel or framework for using these markers in inhalation injury prognostication. While the identified DE miRNAs were supported using an additional bioinformatics tool, strict filtering and pathway enrichment tools, further validation with RT-qPCR would enhance reliability and establish a definitive miRNA panel. Once a validated miRNA panel is established and its pathway involvement confirmed, *in vitro* and *vivo* studies utilizing miRNA mimics and inhibitors could provide deeper insight into their direct and indirect roles in burns-related inhalation injury pathology. This approach would also help distinguish miRNA-mediated effects from secondary molecular interactions within exosomal cargo. Further

research should also investigate proteogenomic expression differences between inhalation injury and underlying or related pulmonary conditions that may complicate the clinical presentation.

REFERENCES

Abd-El-Fattah, A.A., Sadik, N.A.H., Shaker, O.G. & Aboulftouh, M.L. 2013. Differential microRNAs expression in serum of patients with lung cancer, pulmonary tuberculosis, and Pneumonia. *Cell Biochemistry & Biophysics*, 67(3): 875–884.

Abdi, S., Herndon, D.N., Traber, L.D., Ashley, K.D., Stothert, J.C., Maguire, J., Butler, R. & Traber, D.L. 1991. Lung edema formation following inhalation injury: role of the bronchial blood flow. *Journal of Applied Physiology*, 71(2): 727–734.

Afshar, M., Burnham, E.L., Joyce, C., Gagnon, R., Dunn, R., Albright, J.M., Ramirez, L., Repine, J.E., Netzer, G. & Kovacs, E.J. 2019. Injury characteristics and von Willebrand factor for the prediction of acute respiratory distress syndrome in patients with burn injury. *Annals of Surgery*, 270(6): 1186–1193.

Agbenorku, P., Agbenorku, M., Bayuo, J., Yaa, N. & Asare, O. 2019. Epidemiology and outcome of suspected inhalational burn injury in a Ghanaian tertiary hospital. *Burns Open*, 3(2): 45–50.

Ageeva, T., Rizvanov, A. & Mukhamedshina, Y. 2024. NF-κB and JAK/STAT signaling pathways as crucial regulators of neuroinflammation and astrocyte modulation in spinal cord injury. *Cells*, 13(7): 581.

Ahn, C. & Maitz, P. 2012. The true cost of burn. *Burns*, 38(7): 967–974.

Akarachantachote, N., Chadcham, S. & Saithanu, K. 2014. Cutoff threshold of variable importance in projection for variable selection. *International Journal of Pure & Apllied Mathematics*, 94(3): 307-322.

Akhter, M.S., Uddin, M.A. & Barabutis, N. 2020. P53 Regulates the Redox Status of Lung Endothelial Cells. *Inflammation*, 43(2): 686–691.

Al-Shaqsi, S., Al-Kashmiri, A. & Al-Bulushi, T. 2013. Epidemiology of burns undergoing hospitalization to the National Burns Unit in the Sultanate of Oman: A 25-year review. *Burns*, 39(8): 1606–1611.

Albertine, K.H., Soulier, M.F., Wang, Z., Ishizaka, A., Hashimoto, S., Zimmerman, G.A., Matthay, M.A. & Ware, L.B. 2002. Fas and Fas ligand are up-regulated in pulmonary edema fluid and lung tissue of patients with acute lung injury and the acute respiratory distress syndrome. *The American Journal of Pathology*, 161(5): 1783–1796.

Albright, J.M., Davis, C.S., Bird, M.D., Ramirez, L., Kim, H., Burnham, E.L., Gamelli, R.L. & Kovacs, E.J. 2012. The acute pulmonary inflammatory response to the graded severity of smoke inhalation injury. *Critical Care Medicine*, 40(4): 1113–1121.

Alden, N.E., Bessey, P.Q., Rabbitts, A., Hyden, P. & Yurt, R.W. 2006. Tap Water Scalds Among Seniors and the Elderly: Socioeconomics and implications for prevention. *Journal of Burn Care & Research*, 27(Supplement): S97.

Al Ashry, H.S., Mansour, G., Kalil, A.C., Walters, R.W. & Vivekanandan, R. 2016. Incidence of ventilator associated pneumonia in burn patients with inhalation injury treated with high frequency percussive ventilation versus volume control ventilation: A systematic review. *Burns*, 42(6): 1193–1200.

Ali, Meher B & Ali, Mashal B. 2022. Psychological and physiological complications of post-burn patients in Pakistan. *Sultan Qaboos University Medical Journal*, 22(1): 8–13.

Allorto, N.L., Oosthuizen, G. V, Clarke, D.L. & Muckart, D.J. 2009. The spectrum and outcome of burns at a regional hospital in South Africa. *Burns*, 35(7): 1004–1008.

Allorto, N.L., Wall, S. & Clarke, D.L. 2018. Quantifying capacity for burn care in South Africa. *Burns Open*, 2(4): 188–192.

Allorto, N.L., Zoepke, S., Clarke, D.L. & Rode, H. 2016. Burn surgeons in South Africa: A rare species. *South African Medical Journal*, 106(2): 186–188.

Alp, E., Coruh, A., Gunay, G.K., Yontar, Y. & Doganay, M. 2012. Risk factors for nosocomial infection and mortality in burn patients: 10 years of experience at a university hospital. *Journal of Burn Care & Research*, 33(3): 379–385.

Alshiakh, S.M. 2023. Role of serum lactate as prognostic marker of mortality among emergency department patients with multiple conditions: A systematic review. *SAGE Open Medicine*, 11: 205031212211364.

Altuvia, Y., Landgraf, P., Lithwick, G., Elefant, N., Pfeffer, S., Aravin, A., Brownstein, M.J., Tuschl, T. & Margalit, H. 2005. Clustering and conservation patterns of human microRNAs. *Nucleic Acids Research*, 33(8): 2697–2706.

Alunni-Fabbroni, M., Majunke, L., Trapp, E.K., Tzschaschel, M., Mahner, S., Fasching, P.A., Fehm, T., Schneeweiss, A., Beck, T., Lorenz, R., Friedl, T.W.P., Janni, W., Rack, B. & Group, S.S. 2018. Whole blood microRNAs as potential biomarkers in post-operative early breast cancer patients. *BioMed Central cancer*, 18(1): 141.

Ambros, V. 2001. MicroRNAs: Tiny regulators with great potential. *Cell*, 107: 823–826.

Ambros, V., Bartel, B., Bartel, D.P., Burge, C.B., Carrington, J.C., Chen, X., Dreyfuss, G., Eddy, S.R., Griffiths-jones, S. a M., Marshall, M., Matzke, M., Ruvkun, G. & Tuschl, T. 2003. A uniform system for microRNA annotation. *RNA*, 9(3): 277–279.

American Burn Association. 2019. *National Burn Repository 2019 Update*. http://ameriburn.org/wp-content/uploads/2019/02/2017_aba_nbr_annual_report.pdf [20 September 2019]

Anastas, J.N. & Moon, R.T. 2013. WNT signalling pathways as therapeutic targets in cancer. *Nature Reviews Cancer*, 13(1): 11–26.

Ancel, L., Gabillot, O., Szurewsky, C., Granger, R., Sache, A., Voyer, F., Gruel, G., Illiano, S., Benderitter, M., Le Guen, B., Souidi, M., Benadjaoud, M.A. & Flamant, S. 2024. microRNA blood signature for localized radiation injury. *Scientific Reports*, 14(1): 2681.

Andrews, S. 2010. *FastQC: a quality control tool for high throughput sequence data*. http://www.bioinformatics.babraham.ac.uk/projects/fastqc [11 November 2023].

Anfossi, S., Giordano, A., Gao, H., Cohen, E.N., Tin, S., Wu, Q., Garza, R.J., Debeb, B.G., Alvarez, R.H., Valero, V., Hortobagyi, G.N., Calin, G.A., Ueno, N.T., Woodward, W.A. & Reuben, J.M. 2014. High serum miR-19a levels are associated with inflammatory breast cancer and are predictive of favorable clinical outcome in patients with metastatic HER2+ inflammatory breast cancer. *PLoS One*, 9(1): e83113–e83113.

Angelou, I.K., van Aswegen, H., Wilson, M. & Grobler, R. 2022. A profile of adult patients with major burns admitted to a Level 1 Trauma Centre and their functional outcomes at discharge: A retrospective review. *South African Journal of Physiotherapy*, 78(1): 1543.

Angulo, M., Lecuona, E. & Sznajder, J.I. 2012. Rol de los microARN en las enfermedades pulmonares. *Archivos de Bronconeumología*, 48(9): 325–330.

Anous, M.M. & Heimbach, D.M. 1986. Causes of death and predictors in burned patients more than 60 years of age. *The Journal of Trauma*, 26(2): 135–139.

Antonio, A.C.P., Castro, P.S. & Freire, L.O. 2013. Smoke inhalation injury during enclosed-space fires: an update. *Jornal Brasileiro de Pneumologia*, 39(3): 373–381.

Aparicio-Puerta, E., Gómez-Martín, C., Giannoukakos, S., Medina, J.M., Scheepbouwer, C., García-Moreno, A., Carmona-Saez, P., Fromm, B., Pegtel, M., Keller, A., Marchal, J.A. & Hackenberg, M. 2022. sRNAbench and sRNAtoolbox 2022 update: accurate miRNA and sncRNA profiling for model and non-model organisms. *Nucleic Acids Research*, 50(W1): W710–W717.

Arroyo, J., Chevillet, J., Kroh, E., Ruf, I., Pritchard, C., Gibson, D., Mitchell, P., Bennett, C., Pogosova-Agadjanyan, E., Stirewalt, D., Tait, J. & Tewari, M. 2011. Argonaute2 complexes carry a population of circulating microRNAs independent of vesicles in human plasma. *Proceedings of the National Academy of Sciences of the United States of America*, 108(12): 5003–5008.

Ashburner, M., Ball, C.A., Blake, J.A., Botstein, D., Butler, H., Cherry, J.M., Davis, A.P., Dolinski, K., Dwight, S.S., Eppig, J.T., Harris, M.A., Hill, D.P., Issel-Tarver, L., Kasarskis, A., Lewis, S., Matese, J.C., Richardson, J.E., Ringwald, M., Rubin, G.M. & Sherlock, G. 2000. Gene Ontology: tool for the unification of biology. *Nature Genetics*, 25(1): 25–29.

Atarod, S., Smith, H., Dickinson, A. & Wang, X.-N. 2014. MicroRNA levels quantified in whole blood varies from PBMCs. *F1000Research*, 3: 183.

Atiyeh, B., Masellis, A. & Conte, C. 2009. Optimizing burn treatment in developing low-and middle-income countries with limited health care resources (Part 2). *Annals of Burns and Fire Disasters*, 22(4): 189–195.

Atkinson, G., Watson, P., Maughan, R.J., Shirreffs, S.M. & Nevill, A.M. 2004. A spurious correlation. *Journal of Applied Physiology*, 97(2): 792–793.

Bader, G.D. & Hogue, C.W. 2003. An automated method for finding molecular complexes in large protein interaction networks. *BioMed Central Bioinformatics*, 4(1): 2.

Bae, S., Park, P.S.U., Lee, Y., Mun, S.H., Giannopoulou, E., Fujii, T., Lee, K.P., Violante, S.N., Cross, J.R. & Park-Min, K.-H. 2021. MYC-mediated early glycolysis negatively regulates proinflammatory responses by controlling IRF4 in inflammatory macrophages. *Cell Reports*, 35(11): 109264.

Baeuerle, P.A. & Henkel, T. 1994. Function and Activation of NF-kappaB in the Immune System. *Annual Review of Immunology*, 12(1): 141–179.

Baile, E.M., Dahlby, R.W., Wiggs, B.R. & Pare, P.D. 1985. Role of tracheal and bronchial circulation in respiratory heat exchange. *Journal of Applied Physiology*, 58(1): 217–222.

Barabutis, N. 2020. P53 in RhoA regulation. *Cytoskeleton*, 77(5–6): 197–201.

Barabutis, N. 2019. Unfolded protein response in acute respiratory distress syndrome. *Lung*, 197(6): 827–828.

Barabutis, N., Schally, A. V & Siejka, A. 2018. P53, GHRH, inflammation and cancer. *eBioMedicine*, 37: 557–562.

Bartel, D.P. 2009. MicroRNAs: Target Recognition and Regulatory Functions. *Cell*, 136(2): 215–233.

Bartel, D.P. 2004. MicroRNAs. *Cell*, 116(2): 281–297.

Baud, F.J., Borron, S.W., Mégarbane, B., Trout, H., Lapostolle, F., Vicaut, E., Debray, M. & Bismuth, C. 2002. Value of lactic acidosis in the assessment of the severity of acute cyanide poisoning. *Critical Care Medicine*, 30(9): 2044–2050.

Bauling, P.C., Kruger, J.S., van der Vyver, J.D., van Rooyen, M. & Lombard, J.H. 1992. Early benefits of improved burn-care capability at Kalafong Hospital. *South African Medical Journal*, 81(6): 310–312.

Baux, S. 1961. *Contribution a l'Etude du Traitement Local des Brulures Thermigues Etendues*.

Published PhD thesis, Université de Paris (1896-1968), Paris, France: AGEMP.

Becker, C., Riedmaier, I. & Pfaffl, M.W. 2010. mRNA and microRNA quality control for RT-qPCR analysis. *Methods*, 50(4): 237–243.

Behm-Ansmant, I., Rehwinkel, J., Doerks, T., Stark, A., Bork, P. & Izaurralde, E. 2006. mRNA degradation by miRNAs and GW182 requires both CCR4:NOT deadenylase and DCP1:DCP2 decapping complexes. *Genes & Development*, 20(14): 1885–1898.

Belgian Outcome in Burn Injury Study Group. 2009. Development and validation of a model for prediction of mortality in patients with acute burn injury. *British Journal of Surgery*, 96(1): 111–117.

Benes, V. & Castoldi, M. 2010. Expression profiling of microRNA using real-time quantitative PCR, how to use it and what is available. *Methods*, 50(4): 244–249.

Benmeir, P., Sagi, A., Greber, B., Vardy, D., Lusthaus, S., Picard, E., Baruchin, A., Hauben, D., Ben-Yaqar, Y., Rosenberg, L. & Mahler, D. 1991. An analysis of mortality in patients with burns covering 40 per cent BSA or more: a retrospective review covering 24 years (1964–88). *Burns*, 17(5): 402–405.

Beresneva, E.A., Smirnov, S., Sheviakova, S. V., Spiridonova, T.G. & Barinova, M. 2014. *X-ray findings and morphologic changes in lungs after thermal injury*. *Proceedings of the 2014 European Congress of Radiology, Vienna, 6-10 March 2014.* Austria: ESR: B-0878.

Poster number B-0878.

Bhaskaran, M. & Mohan, M. 2014. MicroRNAs: History, biogenesis, and their evolving role in animal development and disease. *Veterinary Pathology*, 51(4): 759–774.

Bhatia, M. & Moochhala, S. 2004. Role of inflammatory mediators in the pathophysiology of acute respiratory distress syndrome. *The Journal of Pathology*, 202(2): 145–156.

Bianchi, F., Nicassio, F., Marzi, M., Belloni, E., Dall'olio, V., Bernard, L., Pelosi, G., Maisonneuve, P., Veronesi, G. & Di Fiore, P.P. 2011. A serum circulating miRNA diagnostic test to identify asymptomatic high-risk individuals with early stage lung cancer. *European Molecular Biology Organization Molecular Medicine*, 3(8): 495–503.

Bilan, N., Dastranji, A. & Ghalehgolab Behbahani, A. 2015. Comparison of the spo2/fio2 ratio and the pao2/fio2 ratio in patients with acute lung injury or acute respiratory distress syndrome. *Journal of Cardiovascular and Thoracic Research*, 7(1): 28–31.

Bingham, H.G., Gallagher, T.J. & Powell, M.D. 1987. Early bronchoscopy as a predictor of ventilatory support for burned patients. *Journal of Trauma*, 27(11): 1286–1288.

Bisgin, H., Gong, B., Wang, Y. & Tong, W. 2018. Evaluation of bioinformatics approaches for next-generation sequencing analysis of microRNAs with a toxicogenomics study design. *Frontiers in Genetics*, 9: 22.

Bittner, E. & Sheridan, R. 2023. Acute respiratory distress syndrome, mechanical ventilation, and inhalation injury in burn patients. *Surgical Clinics*, 103(3): 439–451.

Blom, L., Klingberg, A., Laflamme, L., Wallis, L. & Hasselberg, M. 2016. Gender differences in burns: A study from emergency centres in the Western Cape, South Africa. *Burns*, 42(7): 1600–1608.

Blom, L., van Niekerk, A. & Laflamme, L. 2011. Epidemiology of fatal burns in rural South Africa: A mortuary register-based study from Mpumalanga Province. *Burns*, 37(8): 1394–1402.

Blumenthal, R. 2009. A retrospective descriptive study of electrocution deaths in Gauteng, South Africa: 2001–2004. *Burns*, 35(6): 888–894.

Bock, F.J. & Tait, S.W.G. 2020. Mitochondria as multifaceted regulators of cell death. *Nature Reviews Molecular Cell Biology*, 21(2): 85–100.

Boissin, C., Hasselberg, M., Kronblad, E., Kim, S.-M., Wallis, L., Rode, H. & Laflamme, L. 2017. Adherence to referral criteria at admission and patient management at a specialized burns centre: The case of the Red Cross War Memorial Children's Hospital in Cape Town, South Africa. *International Journal of Environmental Research & Public Health*, 14(7): 732.

Boissin, C., Wallis, L.A., Kleintjes, W. & Laflamme, L. 2019. Admission factors associated with the in-hospital mortality of burns patients in resource-constrained settings: A two-year retrospective investigation in a South African adult burns centre. *Burns*, 45(6): 1462–1470.

Bollati, V. & Dioni, L. 2019. Methods for analyzing miRNA expression. In McCullough, S.D. & Dolinoy, D. C. B. T.-T. (eds.) *Toxicoepigenetics: Core principles and applications*. London, UK: Elsevier Academic Press: 379–405.

Boon, R.A. & Vickers, K.C. 2013. Intercellular transport of microRNAs. *Arteriosclerosis, Thrombosis & Vascular Biology*, 33(2): 186–192.

Bordon, J., Aliberti, S., Fernandez-Botran, R., Uriarte, S.M., Rane, M.J., Duvvuri, P., Peyrani, P., Morlacchi, L.C., Blasi, F. & Ramirez, J.A. 2013. Understanding the roles of cytokines and neutrophil activity and neutrophil apoptosis in the protective versus deleterious inflammatory response in pneumonia. *International Journal of Infectious Diseases*, 17(2): e76–e83.

Bowman, T., Broome, M.A., Sinibaldi, D., Wharton, W., Pledger, W.J., Sedivy, J.M., Irby, R., Yeatman, T., Courtneidge, S.A. & Jove, R. 2001. Stat3-mediated Myc expression is required for Src transformation and PDGF-induced mitogenesis. *Proceedings of the National Academy of Sciences*, 98(13): 7319–7324.

Brain, S.D., Williams, T.J., Tippins, J.R., Morris, H.R. & MacIntyre, I. 1985. Calcitonin gene-related peptide is a potent vasodilator. *Nature*, 313(5997): 54–56.

Brembeck, F.H., Rosário, M. & Birchmeier, W. 2006. Balancing cell adhesion and Wnt signaling, the key role of β-catenin. *Current Opinion in Genetics & Development*, 16(1): 51–59.

Brennecke, J., Stark, A., Russell, R.B. & Cohen, S.M. 2005. Principles of MicroRNA–Target Recognition. *PLoS Biology*, 3(3): e85.

Brooks, G.A. 2009. Cell–cell and intracellular lactate shuttles. *The Journal of Physiology*, 587(23): 5591–5600.

Brooks, G.A. 2000. Intra- and extra-cellular lactate shuttles. *Medicine & Science in Sports & Exercise*, 32(4): 790–799.

Brooks, G.A. 2002. Lactate shuttles in Nature. *Biochemical Society Transactions*, 30(2): 258–264.

Brusselaers, N., Agbenorku, P. & Hoyte-Williams, P.E. 2013. Assessment of mortality prediction models in a Ghanaian burn population. *Burns*, 39(5): 997–1003.

Brusselaers, N., Hoste, E., Monstrey, S., Colpaert, K., De Waele, J., Vandewoude, K. & Blot, S. 2005. Outcome and changes over time in survival following severe burns from 1985 to 2004. *Intensive Care Medicine*, 31(12): 1648–1653.

Brusselaers, N, Juhász, I., Erdei, I., Monstrey, S. & Blot, S. 2009a. Evaluation of mortality following severe burns injury in Hungary: External validation of a prediction model developed on Belgian burn data. *Burns*, 35(7): 1009–1014.

Brusselaers, N., Monstrey, S. & Blot, S. 2009b. The FLAMES score accurately predicts mortality risk in burn patients (Gomez M, et al. 2008). *Journal of Trauma*, 67(2): 415.

Brusselaers, N., Monstrey, S., Vogelaers, D., Hoste, E. & Blot, S. 2010. Severe burn injury in europe:

a systematic review of the incidence, etiology, morbidity, and mortality. *Critical Care*, 14(5): R188.

Buermans, H.P.J. & den Dunnen, J.T. 2014. Next generation sequencing technology: Advances and applications. *Biochimica et Biophysica Acta*, 1842(10): 1932–1941.

Bull, J.P. 1971. Revised analysis of mortality due to burns. *The Lancet*, 298(7734): 1133–1134.

Bull, J.P. & Squire, J.R. 1949. A study of mortality in a burns unit: Standards for the evaluation of alternative methods of treatment. *Annals of Surgery*, 130(2): 160–173.

Burns, G.P. 2014. Arterial blood gases made easy. *Clinical Medicine*, 14(1): 66–68.

Cancio, L.C. 2009. Airway management and smoke inhalation injury in the burn patient. *Clinics in Plastic Surgery*, 36(4): 555–567.

Cancio, L.C., Barillo, D.J., Kearns, R.D., Holmes, J.H., Conlon, K.M., Matherly, A.F., Cairns, B.A., Hickerson, W.L. & Palmieri, T. 2017. Guidelines for burn care under austere conditions. *Journal of Burn Care & Research*, 38(4): 203–214.

Cao, X., Cai, Z., Liu, J., Zhao, Y., Wang, X., Li, X. & Xia, H. 2017. MiRNA-504 inhibits p53-dependent vascular smooth muscle cell apoptosis and may prevent aneurysm formation. *Molecular Medicine Reports*, 16(3): 2570–2578.

Cao, Y., Wang, H., Yang, L., Zhang, Z., Li, C., Yuan, X., Bu, L., Chen, L., Chen, Y., Li, C.-M. & Guo, D. 2018. PTEN-L promotes type I interferon responses and antiviral immunity. *Cellular & Molecular Immunology*, 15(1): 48–57.

Carbon, S., Douglass, E., Good, B.M., Unni, D.R., Harris, N.L., Mungall, C.J., Basu, S., Chisholm, R.L., Dodson, R.J., Hartline, E., Fey, P., Thomas, P.D., Albou, L.-P., Ebert, D., Kesling, M.J., Mi, H., Muruganujan, A., Huang, X., Mushayahama, T., LaBonte, S.A., Siegele, D.A., Antonazzo, G., Attrill, H., Brown, N.H., Garapati, P., Marygold, S.J., Trovisco, V., dos Santos, G., Falls, K., Tabone, C., Zhou, P., Goodman, J.L., Strelets, V.B., Thurmond, J., Garmiri, P., Ishtiaq, R., Rodríguez-López, M., Acencio, M.L., Kuiper, M., Lægreid, A., Logie, C., Lovering, R.C., Kramarz, B., Saverimuttu, S.C.C., Pinheiro, S.M., Gunn, H., Su, R., Thurlow, K.E., Chibucos, M., Giglio, M., Nadendla, S., Munro, J., Jackson, R., Duesbury, M.J., Del-Toro, N., Meldal, B.H.M., Paneerselvam, K., Perfetto, L., Porras, P., Orchard, S., Shrivastava, A., Chang, H.-Y., Finn, R.D., Mitchell, A.L., Rawlings, N.D., Richardson, L., Sangrador-Vegas, A., Blake, J.A., Christie, K.R., Dolan, M.E., Drabkin, H.J., Hill, D.P., Ni, L., Sitnikov, D.M., Harris, M.A., Oliver, S.G., Rutherford, K., Wood, V., Hayles, J., Bähler, J., Bolton, E.R., De Pons, J.L., Dwinell, M.R., Hayman, G.T., Kaldunski, M.L., Kwitek, A.E., Laulederkind, S.J.F., Plasterer, C., Tutaj, M.A., Vedi, M., Wang, S.-J., D'Eustachio, P., Matthews, L., Balhoff, J.P., Aleksander, S.A., Alexander, M.J., Cherry, J.M., Engel, S.R., Gondwe, F., Karra, K., Miyasato, S.R., Nash, R.S., Simison, M., Skrzypek, M.S., Weng, S., Wong, E.D., Feuermann, M., Gaudet, P., Morgat, A., Bakker, E., Berardini, T.Z., Reiser, L., Subramaniam, S., Huala, E., Arighi, C.N., Auchincloss, A., Axelsen, K., Argoud-Puy, G., Bateman, A., Blatter, M.-C., Boutet, E., Bowler, E., Breuza, L., Bridge, A., Britto, R., Bye-A-Jee, H., Casas, C.C., Coudert, E., Denny, P., Estreicher, A., Famiglietti, M.L., Georghiou, G., Gos, A., Gruaz-Gumowski, N., Hatton-Ellis, E., Hulo, C., Ignatchenko, A., Jungo, F., Laiho, K., Le Mercier, P., Lieberherr, D., Lock, A., Lussi, Y., MacDougall, A., Magrane, M., Martin, M.J., Masson, P., Natale, D.A., Hyka-Nouspikel, N., Orchard, S., Pedruzzi, I., Pourcel, L., Poux, S., Pundir, S., Rivoire, C., Speretta, E., Sundaram, S., Tyagi, N., Warner, K., Zaru, R., Wu, C.H., Diehl, A.D., Chan, J.N., Grove, C., Lee, R.Y.N., Muller, H.-M., Raciti, D., Van Auken, K., Sternberg, P.W., Berriman, M., Paulini, M., Howe, K., Gao, S., Wright, A., Stein, L., Howe, D.G., Toro, S., Westerfield, M., Jaiswal, P., Cooper, L. & Elser, J. 2021. The Gene Ontology resource: enriching a GOld mine. *Nucleic Acids Research*, 49(D1): D325–D334.

Carpenter, R.E., Tamrakar, V., Chahar, H., Vine, T. & Sharma, R. 2022. Confirming multiplex RT-qPCR use in COVID-19 with next-generation sequencing: Strategies for epidemiological

advantage. *Global Health*, 2022: 2270965.

Carr, J.A. & Crowley, N. 2013. Prophylactic sequential bronchoscopy after inhalation injury: results from a three-year prospective randomized trial. *European Journal of Trauma and Emergency Surgery*, 39(2): 177–183.

Carthew, R.W. & Sontheimer, E.J. 2009. Origins and mechanisms of miRNAs and siRNAs. *Cell*, 136(4): 642–655.

Casper, J.K., Clark, W.R., Kelley, R.T. & Colton, R.H. 2002. Laryngeal and phonatory status after burn/inhalation injury: A long term follow-up study. *The Journal of Burn Care & Rehabilitation*, 23(4): 235–243.

Castro, R.C., Gonçales, R.A., Zambuzi, F.A. & Frantz, F.G. 2021. Notch signaling pathway in infectious diseases: role in the regulation of immune response. *Inflammation Research*, 70(3): 261–274.

Catalanotto, C., Cogoni, C. & Zardo, G. 2016. MicroRNA in control of gene expression: An overview of nuclear functions. *International Journal of Molecular Sciences*, 17(10): 1712.

Chan, E.D., Chan, Michael M & Chan, Mallory M. 2013. Pulse oximetry: Understanding its basic principles facilitates appreciation of its limitations. *Respiratory Medicine*, 107(6): 789–799.

Chang, L. & Xia, J. 2023. MicroRNA Regulatory Network Analysis Using miRNet 2.0 BT - Transcription Factor Regulatory Networks. In Q. Song & Z. Tao, eds. *Transcription Factor Regulatory Networks*. New York, NY: Springer US: 185–204.

Chang, L., Zhou, G., Soufan, O. & Xia, J. 2020. miRNet 2.0: network-based visual analytics for miRNA functional analysis and systems biology. *Nucleic Acids Research*, 48(W1): W244–W251.

Charlson, M.E., Pompei, P., Ales, K.L. & MacKenzie, C.R. 1987. A new method of classifying prognostic comorbidity in longitudinal studies: Development and validation. *Journal of Chronic Diseases*, 40(5): 373–383.

Chen, E.Y., Tan, C.M., Kou, Y., Duan, Q., Wang, Z., Meirelles, G.V., Clark, N.R. & Ma'ayan, A. 2013a. Enrichr: interactive and collaborative HTML5 gene list enrichment analysis tool. *BioMed Central Bioinformatics*, 14(1): 128.

Chen, K., Lin, S., Li, P., Song, Q., Luo, D., Liu, T., Zeng, L. & Zhang, W. 2018. Characterization of Staphylococcus aureus isolated from patients with burns in a regional burn center, Southeastern China. *BioMed Central Infectious Diseases*, 18(1): 51.

Chen, K., Zhang, W., Chen, J., Li, S. & Guo, G. 2013b. Rho-associated protein kinase modulates neurite extension by regulating microtubule remodeling and vinculin distribution. *Neural Regeneration Research*, 8(32): 3027–35.

Chen, X., Ba, Y., Ma, L., Cai, X., Yin, Y., Wang, K., Guo, J., Zhang, Yujing, Chen, J., Guo, X., Li, Q., Li, X., Wang, W., Zhang, Yan, Wang, J., Jiang, X., Xiang, Y., Xu, C., Zheng, P. & Zhang, C.-Y. 2008. Characterization of microRNAs in serum: a novel class of biomarkers for diagnosis of cancer and other diseases. *Cell Research*, 18(10): 997–1006.

Chen, Y.-C., Hsu, P.-Y., Su, M.-C., Chin, C.-H., Liou, C.-W., Wang, T.-Y., Lin, Y.-Y., Lee, C.P., Lin, M.-C. & Hsiao, C.-C. 2020. miR-21-5p under-expression in patients with obstructive sleep apnea modulates intermittent hypoxia with re-oxygenation-induced-cell apoptosis and cytotoxicity by targeting pro-inflammatory TNF-α-TLR4 signaling. *International Journal of Molecular Sciences*, 21(3): 999.

Cheng, H.H., Yi, H.S., Kim, Y., Kroh, E.M., Chien, J.W., Eaton, K.D., Goodman, M.T., Tait, J.F., Tewari, M. & Pritchard, C.C. 2013. Plasma processing conditions substantially influence

circulating microRNA biomarker levels. *PLoS One*, 8(6): e64795–e64795.

Cheng, L., Sharples, R.A., Scicluna, B.J. & Hill, A.F. 2014. Exosomes provide a protective and enriched source of miRNA for biomarker profiling compared to intracellular and cell-free blood. *Journal of Extracellular Vesicles*, 3(1): 10.3402/jev.v3.23743.

Cheng, M., Wang, D. & Roussel, M.F. 1999. Expression of c-Myc in response to colony-stimulating factor-1 requires mitogen-activated protein kinase kinase-1. *Journal of Biological Chemistry*, 274(10): 6553–6558.

Chiarugi, A. & Moskowitz, M. 2003. Poly(ADP-ribose) polymerase-1 activity promotes NF-kappaB-driven transcription and microglial activation: implication for neurodegenerative disorders. *Journal of Neurochemistry*, 85(2): 306–317.

Ching, J.A., Shah, J.L., Doran, C.J., Chen, H., Payne, W.G. & Smith Jr, D.J. 2015. The evaluation of physical exam findings in patients assessed for suspected burn inhalation injury. *Journal of Burn Care & Research*, 36(1): 197–202.

Chiurillo, M.A. 2015. Role of the Wnt/β-catenin pathway in gastric cancer: An in-depth literature review. *World Journal of Experimental Medicine*, 5(2): 84.

Choi, H.-R., Ha, J.S., Kim, E.-A., Cho, S.-W. & Yang, S.-J. 2022. MiR-30a-5p and miR-153-3p regulate LPS-induced neuroinflammatory response and neuronal apoptosis by targeting NeuroD1. *BMB Reports*, 55(9): 447–452.

Chopra, M., Reuben, J.S. & Sharma, A.C. 2009. Acute lung injury: Apoptosis and signaling mechanisms. *Experimental Biology & Medicine*, 234(4): 361–371.

Chou, C.H., Shrestha, S., Yang, C.D., Chang, N.W., Lin, Y.L., Liao, K.W., Huang, W.C., Sun, T.H., Tu, S.J., Lee, W.H., Chiew, M.Y., Tai, C.S., Wei, T.Y., Tsai, T.R., Huang, H.T., Wang, C.Y., Wu, H.Y., Ho, S.Y., Chen, P.R., Chuang, C.H., Hsieh, P.J., Wu, Y.S., Chen, W.L., Li, M.J., Wu, Y.C., Huang, X.Y., Ng, F.L., Buddhakosai, W., Huang, P.C., Lan, K.C., Huang, C.Y., Weng, S.L., Cheng, Y.N., Liang, C., Hsu, W.L. & Huang, H. Da. 2018. MiRTarBase update 2018: A resource for experimentally validated microRNA-target interactions. *Nucleic Acids Research*, 46(D1): D296–D302.

Chou, S.H., Lin, S.-D., Chuang, H.-Y., Cheng, Y.-J., Kao, E.L. & Huang, M.-F. 2004. Fiber-optic bronchoscopic classification of inhalation injury: prediction of acute lung injury . *Surgical Endoscopy & Other Interventional Techniques*, 18(9): 1377–1379.

Christopoulos, P.F., Gjølberg, T.T., Krüger, S., Haraldsen, G., Andersen, J.T. & Sundlisæter, E. 2021. Targeting the notch signaling pathway in chronic inflammatory diseases. *Frontiers in Immunology*, 12: 668207.

Chukamei, Z., Mobayen, M., Toolaroud, P., Ghalandari, M. & Delavari, S. 2021. The length of stay and cost of burn patients and the affecting factors. *International Journal of Burns & Trauma*, 11(5): 397–405.

Church, D., Elsayed, S., Reid, O., Winston, B. & Lindsay, R. 2006. Burn wound infections. *Clinical Microbiology Reviews*, 19(2): 403–434.

Cioffi Jr, W.G., Rue III, L.W., Graves, T.A., McManus, W.F., Mason Jr, A.D. & Pruitt Jr, B.A. 1991. Prophylactic use of high-frequency percussive ventilation in patients with inhalation injury. *Annals of surgery*, 213(6): 575–582.

City of Cape Town. 2016. *State of Cape Town report*. https://resource.capetown.gov.za/documentcentre/Documents/City research reports and review/16429 COCT State of Cape Town Report 2016 FINAL.pdf [23 June 2021].

Clark, C.J., Reid, W.H., Gilmour, W.H. & Campbell, D. 1986. Mortality probability in victims of fire

trauma: revised equation to include inhalation injury. *British Medical Journal*, 292(6531): 1303–1305.

Clark, W.R. 1992. Smoke inhalation: Diagnosis and treatment. *World Journal of Surgery*, 16(1): 24–29.

Cloake, T., Haigh, T., Cheshire, J. & Walker, D. 2017. The impact of patient demographics and comorbidities upon burns admitted to Tygerberg Hospital Burns Unit, Western Cape, South Africa. *Burns*, 43(2): 411–416.

Clunes, L.A., Davies, C.M., Coakley, R.D., Aleksandrov, A.A., Henderson, A.G., Zeman, K.L., Worthington, E.N., Gentzsch, M., Kreda, S.M., Cholon, D., Bennett, W.D., Riordan, J.R., Boucher, R.C. & Tarran, R. 2012. Cigarette smoke exposure induces CFTR internalization and insolubility, leading to airway surface liquid dehydration. *Federation of American Societies for Experimental Biology*, 26(2): 533–545.

Cobley, T.D.D., Hart, W.J., Baldwin, D.L. & Burd, D.A.R. 1999. Complete fusion of the vocal cords; an unusual case. *Burns*, 25(4): 361–363.

Cohen, M.A. & Guzzardi, L.J. 1983. Inhalation of products of combustion. *Annals of Emergency Medicine*, 12(10): 628–632.

Coimbra-Costa, D., Alva, N., Duran, M., Carbonell, T. & Rama, R. 2017. Oxidative stress and apoptosis after acute respiratory hypoxia and reoxygenation in rat brain. *Redox Biology*, 12: 216–225.

Colohan, S.M. 2010. Predicting Prognosis in Thermal Burns With Associated Inhalational Injury: A Systematic Review of Prognostic Factors in Adult Burn Victims. *Journal of Burn Care & Research*, 31(4): 529–539.

Concannon, E., Damkat Thomas, L., Kerr, L., Damkat, I., Reddi, B., Greenwood, J.E., Solanki, N.S. & Wagstaff, M.J.D. 2023. Review of Indications for Endotracheal Intubation in Burn Patients with Suspected Inhalational Injury. *European Burn Journal*, 4(2): 163–172.

Costantini, E., Sinjari, B., Di Giovanni, P., Aielli, L., Caputi, S., Muraro, R., Murmura, G. & Reale, M. 2023. TNFα, IL-6, miR-103a-3p, miR-423-5p, miR-23a-3p, miR-15a-5p and miR-223-3p in the crevicular fluid of periodontopathic patients correlate with each other and at different stages of the disease. *Scientific Reports*, 13(1): 126.

Cook, N. 1998. Methicillin-resistant Staphylococcus aureus versus the burn patient. *Burns*, 24(2): 91–98.

Cooks, T. & Harris, C.C. 2014. p53 Mutations and inflammation-associated cancer are linked through TNF signaling. *Molecular Cell*, 56(5): 611–612.

Cooks, T., Pateras, I.S., Tarcic, O., Solomon, H., Schetter, A.J., Wilder, S., Lozano, G., Pikarsky, E., Forshew, T., Rozenfeld, N., Harpaz, N., Itzkowitz, S., Harris, C.C., Rotter, V., Gorgoulis, V.G. & Oren, M. 2013. Mutant p53 prolongs NF-κB activation and promotes chronic inflammation and inflammation-associated colorectal cancer. *Cancer Cell*, 23(5): 634–646.

Correia, C.N., Nalpas, N.C., McLoughlin, K.E., Browne, J.A., Gordon, S. V, MacHugh, D.E. & Shaughnessy, R.G. 2017. Circulating microRNAs as potential biomarkers of infectious disease. *Frontiers in Immunology*, 8(Suppl 1): 118.

Cortez, M. & Calin, G. 2009. MicroRNA identification in plasma and serum: A new tool to diagnose and monitor diseases. *Expert Opinion on Biological Therapy*, 9(6): 703–711.

Cortez, M.A., Bueso-Ramos, C., Ferdin, J., Lopez-Berestein, G., Sood, A.K. & Calin, G.A. 2011. MicroRNAs in body fluids--the mix of hormones and biomarkers. *Nature Reviews. Clinical Oncology*, 8(8): 467–477.

Coudroy, R., Chen, L., Pham, T., Piraino, T., Telias, I. & Brochard, L. 2019. Acute Respiratory Distress Syndrome: Respiratory Monitoring and Pulmonary Physiology. *Seminars in Respiratory & Critical Care Medicine*, 40(01): 066–080.

Coussens, L.M. & Werb, Z. 2002. Inflammation and cancer. *Nature*, 420(6917): 860–867.

Cox, R.A., Burke, A.S., Jacob, S., Oliveras, G., Murakami, K., Shimoda, K., Enkhbaatar, P., Traber, L.D., Herndon, D.N., Traber, D.L. & Hawkins, H.K. 2009. Activated nuclear factor kappa B and airway inflammation after smoke inhalation and burn injury in sheep. *Journal of Burn Care & Research*, 30(3): 489–498.

Cox, R.A., Burke, A.S., Soejima, K., Murakami, K., Katahira, J., Traber, L.D., Herndon, D.N., Schmalstieg, F.C., Traber, D.L. & Hawkins, H.K. 2003. Airway Obstruction in Sheep with Burn and Smoke Inhalation Injuries. *American Journal of Respiratory Cell & Molecular Biology*, 29(3): 295–302.

Crapo, R.O. 1981. Smoke-inhalation injuries. *Journal of American Medical Association*, 246(15): 1694–1696.

Daffue, B., Moolman, D., Ferreira, S., Roos, L., Schoeman, L., Smit, S. & Joubert, G. 2018. The causes of burn wounds among adult patients treated at Pelonomi Tertiary Hospital, Bloemfontein. *South African Journal of Surgery*, 56: 31–36.

Dahal, P., Ghimire, S., Maharjan, N. & Rai, S. 2016. Baux's and abbreviated burn severity score for the prediction of mortality in patients with acute burn injury. *Journal of College of Medical Sciences-Nepal*, 11(4): 24.

Dancey, C.P. & Reidy, J. 2007. *Statistics without maths for psychology*. Harlow, England: Pearson Education/Prentice Hall: 619.

Dancey, D.R., Hayes, J., Gomez, M., Schouten, D., Fish, J., Peters, W., Slutsky, A.S. & Stewart, T.E. 1999. ARDS in patients with thermal injury. *Intensive Care Medicine*, 25(11): 1231–1236.

Dasari, H., Kumar, A. & Sharma, B. 2008. Burns septicemia- the leading cause of burn mortality. *Journal of Punjab Academy of Forensic Medicine & Toxicology*, 8(2): 10–16.

Dash, B.P., Freischmidt, A., Helferich, A.M., Ludolph, A.C., Andersen, P.M., Weishaupt, J.H. & Hermann, A. 2024. Upregulated miR-10b-5p as a potential miRNA signature in amyotrophic lateral sclerosis patients. *Frontiers in Cellular Neuroscience*, 18: 1457704.

Datta, N., Johnson, C., Kao, D., Gurnani, P., Alexander, C., Polytarchou, C. & Monaghan, T.M. 2023. MicroRNA-based therapeutics for inflammatory disorders of the microbiota-gut-brain axis. *Pharmacological Research*, 194: 106870.

Dauer, D.J., Ferraro, B., Song, L., Yu, B., Mora, L., Buettner, R., Enkemann, S., Jove, R. & Haura, E.B. 2005. Stat3 regulates genes common to both wound healing and cancer. *Oncogene*, 24(21): 3397–3408.

Davé, D.R., Nagarjan, N., Canner, J.K., Kushner, A.L. & Stewart, B.T. 2018. Rethinking burns for low & middle-income countries: Differing patterns of burn epidemiology, care seeking behavior, and outcomes across four countries. *Burns*, 44(5): 1228–1234.

Davis, C.S., Albright, J.M., Carter, S.R., Ramirez, L., Kim, H., Gamelli, R.L. & Kovacs, E.J. 2012. Early pulmonary immune hyporesponsiveness is associated with mortality after burn and smoke inhalation injury. *Journal of Burn Care & Research*, 33(1): 26–35.

Davis, C.S., Janus, S.E., Mosier, M.J., Carter, S.R., Gibbs, J.T., Ramirez, L., Gamelli, R.L. & Kovacs, E.J. 2013. Inhalation injury severity and systemic immune perturbations in burned adults. *Annals of surgery*, 257(6): 1137–1146.

Day, C. & Gray, A. 2003. Health and related indicators. In Ijumba, P. Ntuli, A. & Barron, P. (eds.) *South African Health Review 2002*. Durban: Health Systems Trust.

De, S., Dermawan, J.K.T. & Stark, G.R. 2014. EGF receptor uses SOS1 to drive constitutive activation of NFκB in cancer cells. *Proceedings of the National Academy of Sciences*, 111(32): 11721–11726.

De Backer, D., Creteur, J., Zhang, H., Norrenberg, M. & Vincent, J.-L. 1997. Lactate Production by the Lungs in Acute Lung Injury. *American Journal of Respiratory & Critical Care Medicine*, 156(4): 1099–1104.

De Guire, V., Robitaille, R., Tétreault, N., Guérin, R., Ménard, C., Bambace, N. & Sapieha, P. 2013. Circulating miRNAs as sensitive and specific biomarkers for the diagnosis and monitoring of human diseases: Promises and challenges. *Clinical Biochemistry*, 46(10): 846–860.

De La Cal, M.A., Cerdá, E., García-Hierro, P., Lorente, L., Sánchez-Concheiro, M., Díaz, C. & van Saene, H.K. 2001. Pneumonia in patients with severe burns: a classification according to the concept of the carrier state. *Chest*, 119(4): 1160–1165.

De Lucas, E. H, Sanchez-Sanchez, M., Cachafeiro Fuciños, L., Agrifoglio Rotaeche, A., Martínez Mendez, J.R., Flores Cabeza, E., Millan Estañ, P. & García-de-Lorenzo, A. 2020. Lactate and lactate clearance in critically burned patients: usefulness and limitations as a resuscitation guide and as a prognostic factor. *Burns*, 46(8): 1839–1847.

Del Re, D.P., Miyamoto, S. & Brown, J.H. 2007. RhoA/Rho kinase up-regulate Bax to activate a mitochondrial death pathway and induce cardiomyocyte apoptosis. *Journal of Biological Chemistry*, 282(11): 8069–8078.

De Sarno, P., Shestopal, S.A., Zmijewska, A.A. & Jope, R.S. 2005. Anti-apoptotic effects of muscarine receptor activation are mediated by rho kinase. *Brain Research*, 1041(1):112-115.

Demedts, I.K., Demoor, T., Bracke, K.R., Joos, G.F. & Brusselle, G.G. 2006. Role of apoptosis in the pathogenesis of COPD and pulmonary emphysema. *Respiratory Research*, 7(1): 53.

Demling, R.H. 2008. Smoke inhalation lung injury: an update. *Eplasty*, 8: e27–e27.

Den Hollander, D., Albert, M., Strand, A. & Hardcastle, T.C. 2014. Epidemiology and referral patterns of burns admitted to the Burns Centre at Inkosi Albert Luthuli Central Hospital, Durban. *Burns*, 40(6): 1201–1208.

Deng, Z., Jia, Y., Liu, H., He, M., Yang, Y., Xiao, W. & Li, Y. 2019. RhoA/ROCK pathway: implication in osteoarthritis and therapeutic targets. *American Journal of Translational Research*, 11(9): 5324–5331.

Denli, A.M., Tops, B.B.J., Plasterk, R.H.A., Ketting, R.F. & Hannon, G.J. 2004. Processing of primary microRNAs by the microprocessor complex. *Nature*, 432(7014): 231–235.

Dennis, G., Sherman, B.T., Hosack, D.A., Yang, J., Gao, W., Lane, H.C. & Lempicki, R.A. 2003. DAVID: Database for Annotation, Visualization, and Integrated Discovery. *Genome Biology*, 4(5): P3.

Department of Health. 2020. *Annual report 2019/2020*. https://www.westerncape.gov.za/assets/annual_report_2019-2020.pdf [14 July 2021].

Desjardins, P.R. & Conklin, D.S. 2011. Microvolume quantitation of nucleic qcids. *Current Protocols in Molecular Biology*, 93(1): A.3J.1-A.3J.16.

Dhopte, A., Bamal, R. & Tiwari, V.K. 2017. A prospective analysis of risk factors for pediatric burn mortality at a tertiary burn center in North India. *Burns & trauma*, 5(1): 30.

Di Lorenzo, A., Fernández-Hernando, C., Cirino, G. & Sessa, W.C. 2009. Akt1 is critical for acute

inflammation and histamine-mediated vascular leakage. *Proceedings of the National Academy of Sciences*, 106(34): 14552–14557.

Di Minin, G., Bellazzo, A., Dal Ferro, M., Chiaruttini, G., Nuzzo, S., Bicciato, S., Piazza, S., Rami, D., Bulla, R., Sommaggio, R., Rosato, A., Del Sal, G. & Collavin, L. 2014. Mutant p53 reprograms TNF signaling in cancer cells through interaction with the tumor suppressor DAB2IP. *Molecular Cell*, 56(5): 617–629.

Diamantopoulos, M.A., Tsiakanikas, P. & Scorilas, A. 2018. Non-coding RNAs : the riddle of the transcriptome and their perspectives in cancer. *Annals of Translational Medicine*, 6(12): 241–258.

Diekstra, A., Bosgoed, E., Rikken, A., van Lier, B., Kamsteeg, E.-J., Tychon, M., Derks, R.C., van Soest, R.A., Mensenkamp, A.R., Scheffer, H., Neveling, K. & Nelen, M.R. 2015. Translating Sanger-based routine DNA diagnostics into generic massive parallel ion semiconductor sequencing. *Clinical Chemistry*, 61(1): 154–162.

Diener, C., Hart, M., Kehl, T., Rheinheimer, S., Ludwig, N., Krammes, L., Pawusch, S., Lenhof, K., Tänzer, T., Schub, D., Sester, M., Walch-Rückheim, B., Keller, A., Lenhof, H.-P. & Meese, E. 2020. Quantitative and time-resolved miRNA pattern of early human T cell activation. *Nucleic Acids Research,* 48(18): 10164–10183.

Ding, H., Lv, Q., Wu, S., Hou, S., Liu, Z., Landén, N.X., Tian, P., Yu, M., Sun, Z. & Fan, H. 2017. Intratracheal instillation of perfluorohexane modulates the pulmonary immune microenvironment by attenuating early inflammatory factors in patients with smoke inhalation injury: A randomized controlled clinical trial. *Journal of Burn Care & Research*, 38(4): 251–259.

Ding, J., Li, X., Khan, S., Zhang, C., Gao, F., Sen, S., Wasylishen, A.R., Zhao, Y., Lozano, G., Koul, D. & Alfred Yung, W.K. 2022. EGFR suppresses p53 function by promoting p53 binding to DNA-PKcs: a noncanonical regulatory axis between EGFR and wild-type p53 in glioblastoma. *Neuro-Oncology*, 24(10): 1712–1725.

Dokter, Meijs, J., Oen, I.M.M.H., van Baar, M.E., van der Vlies, C.H. & Boxma, H. 2014. External validation of the revised Baux score for the prediction of mortality in patients with acute burn injury. *The Journal of Trauma & Acute Care Surgery*, 76(3): 840–845.

Dolp, R., Rehou, S., McCann, M.R. & Jeschke, M.G. 2018. Contributors to the length-of-stay trajectory in burn-injured patients. *Burns*, 44(8): 2011–2017.

Domínguez-Cáceres, M.A., García-Martínez, J.M., Calcabrini, A., González, L., Porque, P.G., León, J. & Martín-Pérez, J. 2004. Prolactin induces c-Myc expression and cell survival through activation of Src/Akt pathway in lymphoid cells. *Oncogene*, 23(44): 7378–7390.

Douglas, H.E., Ratcliffe, A., Sandhu, R. & Anwar, U. 2015. Comparison of mortality prediction models in burns ICU patients in Pinderfields Hospital over 3 years. *Burns*, 41(1): 49–52.

Drakopanagiotakis, F., Xifteri, A., Polychronopoulos, V. & Bouros, D. 2008. Apoptosis in lung injury and fibrosis. *European Respiratory Journal*, 32(6): 1631–1638.

Dries, D.J. & Endorf, F.W. 2013. Inhalation injury: Epidemiology, pathology, treatment strategies. *Scandinavian Journal of Trauma, Resuscitation & Emergency Medicine*, 21(1): 31.

Dufourd, T., Robil, N., Mallet, D., Carcenac, C., Boulet, S., Brishoual, S., Rabois, E., Houeto, J.-L., de la Grange, P. & Carnicella, S. 2019. Plasma or serum? A qualitative study on rodents and humans using high-throughput microRNA sequencing for circulating biomarkers. *Biology Methods and Protocols*, 4(1): 1-10.

Duke, J., Wood, F., Semmens, J., Edgar, D.W., Spilsbury, K. & Rea, S. 2012. An assessment of burn injury hospitalisations of adolescents and young adults in Western Australia, 1983–2008. *Burns*, 38(1): 128–135.

Eastman, A.L., Arnoldo, B.A., Hunt, J.L. & Purdue, G.F. 2010. Pre–burn center management of the burned airway: Do we know enough? *Journal of Burn Care & Research*, 31(5): 701–705.

Eaton, W.O. & Yu, A.P. 1989. Are sex differences in child motor activity level a function of sex differences in maturational status? *Child Development*, 60(4): 1005–1011.

Edelman, D.A., Khan, N., Kempf, K. & White, M.T. 2007. Pneumonia after inhalation injury. *Journal of Burn Care and Research*, 28(2): 241–246.

El Soud, M.A.A., Ali, R.A., Taha, A.A. & Khirfan, S.M. 2019. ABSI scoring system for burns: concerns and modifications in a developing country. *European Journal of Plastic Surgery*, 42(2): 177–182.

El-Helbawy, R.H. & Ghareeb, F.M. 2011. Inhalation injury as a prognostic factor for mortality in burn patients. *Annals of Burns & Fire Disasters*, 24(2): 82–88.

Elkamhawy, A., Hassan, A.H.E., Paik, S., Sup Lee, Y., Lee, H.-H., Shin, J.-S., Lee, K.-T. & Roh, E.J. 2019. EGFR inhibitors from cancer to inflammation: Discovery of 4-fluoro-N-(4-(3-(trifluoromethyl)phenoxy)pyrimidin-5-yl)benzamide as a novel anti-inflammatory EGFR inhibitor. *Bioorganic Chemistry*, 86: 112–118.

Endorf, F. & Gamelli, R. 2007. Inhalation injury, pulmonary perturbations, and fluid resuscitation. *Journal of Burn Care & Research*, 28(1): 80–83.

Enkhbaatar, P., Pruitt Jr, B.A., Suman, O., Mlcak, R., Wolf, S.E., Sakurai, H. & Herndon, D.N. 2016. Pathophysiology, research challenges, and clinical management of smoke inhalation injury. *Lancet*, 388(10052): 1437–1446.

Erickson, S.E., Martin, G.S., Davis, J.L., Matthay, M.A., Eisner, M.D. & Network, N.I.H.N.A. 2009. Recent trends in acute lung injury mortality: 1996-2005. *Critical Care Medicine*, 37(5): 1574–1579.

Eriksson, L., Johansson, E., Kettaneh-Wold, N., Trygg, J., Wikström, C. & Wold, S. 2006. *Multi- and megavariate data analysis : Part I: Basic principles and applications*. 2nd revised and enlarged edited. Umeå Sweden: MKS Umetrics AB: 1-103.

Ernst, A. & Zibrak, J.D. 1998. Carbon monoxide poisoning. *New England Journal of Medicine*, 339(22): 1603–1608.

Espinoza, L.A., Smulson, M.E. & Chen, Z. 2007. Prolonged poly(ADP-ribose) polymerase-1 activity regulates JP-8-induced sustained cytokine expression in alveolar macrophages. *Free Radical Biology & Medicine*, 42(9): 1430–1440.

Esteve, P., Embade, N., Perona, R., Jiménez, B., Peso, L. del, León, J., Arends, M., Miki, T. & Lacal, J.C. 1998. Rho-regulated signals induce apoptosis in vitro and in vivo by a p53-independent, but Bcl2 dependent pathway. *Oncogene*, 17(14): 1855–1869.

Evers, L.H., Bhavsar, D. & Mailänder, P. 2010. The biology of burn injury. *Experimental Dermatology*, 19(9): 777–783.

Ewels, P., Magnusson, M., Lundin, S. & Käller, M. 2016. MultiQC: summarize analysis results for multiple tools and samples in a single report. *Bioinformatics*, 32(19): 3047–3048.

Eyal, A.S., Kemp, M. & Luvhengo, T. 2007. A 10-year audit of burns at Kalafong Hospital. *Burns*, 33(3): 393–395.

Fabbri, M., Garzon, R., Cimmino, A., Liu, Z., Zanesi, N., Callegari, E., Liu, S., Alder, H., Costinean, S., Fernandez-Cymering, C., Volinia, S., Guler, G., Morrison, C.D., Chan, K.K., Marcucci, G., Calin, G.A., Huebner, K. & Croce, C.M. 2007. MicroRNA-29 family reverts aberrant methylation in lung cancer by targeting DNA methyltransferases 3A and 3B. *Proceedings of the*

National Academy of Sciences of the United States of America, 104(40): 15805–15810.

Fan, Y., Mao, R. & Yang, J. 2013. NF-κB and STAT3 signaling pathways collaboratively link inflammation to cancer. *Protein & Cell*, 4(3): 176–185.

Fang-Gang, N., Yang, C., Yu-Xuan, Q., Yan-Hua, R., Wei-Li, D., Cheng, W., Chun-Quan, W. & Guo-An, Z. 2015. Laryngeal morphologic changes and epidemiology in patients with inhalation injury: A retrospective study. *Burns*, 41(6): 1340–1346.

Fang, Y., Zou, L. & He, W. 2021. miR-30a-5p mitigates autophagy by regulating the Beclin-1/ATG16 pathway in renal ischemia/reperfusion injury. *International Journal of Molecular Medicine*, 48(1): 144.

Fauth, M., Hegewald, A.B., Schmitz, L., Krone, D.J. & Saul, M.J. 2019. Validation of extracellular miRNA quantification in blood samples using RT-qPCR. *FASEB BioAdvances*, 1(8): 481–492.

Ferruelo, A., Peñuelas, Ó. & Lorente, J.A. 2018. MicroRNAs as biomarkers of acute lung injury. *Annals of Translational Medicine*, 6(2): 34.

Fiala, A., Slagle, C., Legband, N., Aghabaglou, F., Buesing, K., Borden, M., Harris, S. & Terry, B. 2020. Treatment of a Rat Model of LPS-Induced ARDS via Peritoneal Perfusion of Oxygen Microbubbles. *Journal of Surgical Research*, 246: 450–456.

Filipowicz, W., Bhattacharyya, S.N. & Sonenberg, N. 2008. Mechanisms of post-transcriptional regulation by microRNAs: are the answers in sight? *Nature Reviews Genetics*, 9(2): 102–114.

Finnerty, C.C., Herndon, D.N., Przkora, R., Pereira, C.T., Oliveira, H.M., Queiroz, D.M.M., Rocha, A.M.C. & Jeschke, M.G. 2006. Cytokine expression profile over time in severely burned pediatric patients. *Shock*, 26(1): 13–19.

Finnerty, C.C., Ju, H., Spratt, H., Victor, S., Jeschke, M.G., Hegde, S., Bhavnani, S.K., Luxon, B.A., Brasier, A.R. & Herndon, D.N. 2012. Proteomics improves the prediction of burns mortality: results from regression spline modeling. *Clinical & Translational Science*, 5(3): 243–249.

Fleige, S. & Pfaffl, M.W. 2006. RNA integrity and the effect on the real-time qRT-PCR performance. *Molecular Aspects of Medicine*, 27(2–3): 126–139.

Foncerrada, G., Culnan, D.M., Capek, K.D., González-Trejo, S., Cambiaso-Daniel, J., Woodson, L.C., Herndon, D.N., Finnerty, C.C. & Lee, J.O. 2018. Inhalation injury in the burned patient. *Annals of Plastic Surgery*, 80(3 Suppl 2): S98–S105.

Fontán, J.J.P., Cortright, D.N., Krause, J.E., Velloff, C.R., Karpitskyi, V. V., Carver, T.W., Shapiro, S.D. & Mora, B.N. 2000. Substance P and neurokinin-1 receptor expression by intrinsic airway neurons in the rat. *American Journal of Physiology*, 278: 344–355.

Forjuoh, S.N. 2006. Burns in low- and middle-income countries: a review of available literature on descriptive epidemiology, risk factors, treatment, and prevention. *Burns*, 32(5): 529–537.

Forjuoh, S.N., Guyer, B., Strobino, D.M., Keyl, P.M., Diener-West, M. & Smith, G.S. 1995. Risk factors for childhood burns: a case-control study of Ghanaian children. *Journal of Epidemiology & Community Health*, 49(2): 189–193.

Forster, N.A., Zingg, M., Haile, S.R., Künzi, W., Giovanoli, P. & Guggenheim, M. 2011. 30 years later-does the ABSI need revision? *Burns,* 37(6): 958–963.

Forterre, A., Jalabert, A., Chikh, K., Pesenti, S., Euthine, V., Granjon, A., Errazuriz, E., Lefai, E., Vidal, H. & Rome, S. 2014. Myotube-derived exosomal miRNAs downregulate Sirtuin1 in myoblasts during muscle cell differentiation. *Cell Cycle*, 13(1): 78–89.

Francis, S.M.S., Davidson, M.R., Tan, M.E., Wright, C.M., Clarke, B.E., Duhig, E.E., Bowman, R. V., Hayward, N.K., Fong, K.M. & Yang, I.A. 2014. MicroRNA-34c is associated with emphysema

severity and modulates SERPINE1 expression. *BioMed Central Genomics*, 15(1): 88.

Frans, F.A., Keli, S.O. & Maduro, A.E. 2008. The epidemiology of burns in a medical center in the Caribbean. *Burns*, 34(8): 1142–1148.

Freeman, B. 1994. Free radical chemistry of nitric oxide: Looking at the dark side. *Chest*, 105(3, Supplement): 79S-84S.

Friedl, H.P., Till, G.O., Trentz, O. & Ward, P.A. 1989. Roles of histamine, complement and xanthine oxidase in thermal injury of skin. *The American Journal of Pathology*, 135(1): 203–217.

Friedländer, M.R., Chen, W., Adamidi, C., Maaskola, J., Einspanier, R., Knespel, S. & Rajewsky, N. 2008. Discovering microRNAs from deep sequencing data using miRDeep. *Nature Biotechnology*, 26(4): 407–415.

Friedman, R.C., Farh, K.K.-H., Burge, C.B. & Bartel, D.P. 2009. Most mammalian mRNAs are conserved targets of microRNAs. *Genome Research*, 19(1): 92–105.

Fu, W., Liu, Z., Zhang, J., Shi, Y., Zhao, R. & Zhao, H. 2019. Effect of miR-144-5p on the proliferation, migration, invasion and apoptosis of human umbilical vein endothelial cells by targeting RICTOR and its related mechanisms. *Experimental and Therapeutic Medicine*, 19(3): 1817–1823.

Fu, Y., Zhou, E., Wei, Z., Wang, W., Wang, T., Yang, Z. & Zhang, N. 2014. Cyanidin-3-O-β-glucoside ameliorates lipopolysaccharide-induced acute lung injury by reducing TLR4 recruitment into lipid rafts. *Biochemical Pharmacology*, 90(2): 126–134.

Galani, V., Tatsaki, E., Bai, M., Kitsoulis, P., Lekka, M., Nakos, G. & Kanavaros, P. 2010. The role of apoptosis in the pathophysiology of acute respiratory distress syndrome (ARDS): An up-to-date cell-specific review. *Pathology*, 206(3): 145–150.

Gallo, A., Tandon, M., Alevizos, I. & Illei, G.G. 2012. The majority of microRNAs detectable in serum and saliva is concentrated in exosomes. *PLoS One*, 7(3): e30679–e30679.

Gao, C., Wang, Y., Broaddus, R., Sun, L., Xue, F. & Zhang, W. 2018. Exon 3 mutations of CTNNB1 drive tumorigenesis: a review. *Oncotarget*, 9(4): 5492–5508.

Gao, P., Wang, X., Qian, D., Qin, Z.-X., Jin, J., Xu, Q., Yuan, Q.-Y., Li, X.-J. & Si, L.-Y. 2013. Induction of oxidative stress by oxidized LDL via meprinα-activated epidermal growth factor receptor in macrophages. *Cardiovascular Research*, 97(3): 533–543.

Gao, X., Huang, X., Yang, Q., Zhang, S., Yan, Z., Luo, R., Wang, P., Wang, W., Xie, K. & Gun, S. 2021. MicroRNA-21-5p targets PDCD4 to modulate apoptosis and inflammatory response to Clostridium perfringens beta2 toxin infection in IPEC-J2 cells. *Developmental & Comparative Immunology*, 114: 103849.

García-Díaz, A., Gacto-Sánchez, P., Durán-Romero, A.J., Carrasco-García, S., Ruiz-Moya, A., Molina-Morales, J., Sánchez-Tatay, M.-V., Gómez-Cía, T. & Pereyra-Rodríguez, J.-J. 2022. Pediatric major burns: a monocentric retrospective review of etiology and outcomes (2008–2020). *European Journal of Plastic Surgery*, 45(6): 967–975.

Ge, J., Yao, Y., Jia, H., Li, P. & Sun, W. 2020. Inhibition of miR-21 ameliorates LPS-induced acute lung injury through increasing B cell lymphoma-2 expression. *Innate Immunity*, 26(8): 693–702.

Germann, G., Barthold, U., Lefering, R., Raff, T. & Hartmann, B. 1997. The impact of risk factors and pre-existing conditions on the mortality of burn patients and the precision of predictive admission-scoring systems. *Burns*, 23(3): 195–203.

Gerö, D. & Szabó, C. 2008. Poly(ADP-ribose) polymerase: a new therapeutic target? *Current Opinion in Anesthesiology*, 21(2): 111–121.

Gevaart-Durkin, A., Swart, D. & Chowdhury, Z. 2014. A study of energy-related injuries from hospital admissions among children and adults in South Africa. *Burns*, 40(6): 1209–1218.

Ghai, V. & Wang, K. 2016. Recent progress toward the use of circulating microRNAs as clinical biomarkers. *Archives of Toxicology*, 90(12): 2959–2978.

Giaccia, A.J. & Kastan, M.B. 1998. The complexity of p53 modulation: emerging patterns from divergent signals. *Genes & Development*, 12(19): 2973–2983.

Gicheru, B., Shah, J., Wachira, B., Omuse, G. & Maina, D. 2023. The diagnostic accuracy of an initial point-of-care lactate at the emergency department as a predictor of in-hospital mortality among adult patients with sepsis and septic shock. *Frontiers in Medicine*, 10: 1173286.

Gill, P. & Martin, R. V. 2015. Smoke inhalation injury. *British Journal of Anaesthesia Education*, 15(3): 143–148.

Gillen, A.E., Gosalia, N., Leir, S.-H. & Harris, A. 2011. MicroRNA regulation of expression of the cystic fibrosis transmembrane conductance regulator gene. *The Biochemical Journal*, 438(1): 25–32.

Godinjak, A., Jusufovic, S., Rama, A., Iglica, A., Zvizdic, F., Kukuljac, A., Tancica, I. & Rozajac, S. 2017. Hyperlactatemia and the importance of repeated lactate measurements in critically ill patients. *Medical Archives*, 71(6): 404.

Godwin, Y., Hudson, D.A. & Bloch, C.E. 1997. Shack fires: a consequence of urban migration. *Burns*, 23(2): 151–153.

Godwin, Y. & Wood, S.H. 1998. Major burns in Cape Town: a modified burns score for patient triage. *Burns*, 24(1): 58–63.

Goldie, B.J., Dun, M.D., Lin, M., Smith, N.D., Verrills, N.M., Dayas, C. V & Cairns, M.J. 2014. Activity-associated miRNA are packaged in Map1b-enriched exosomes released from depolarized neurons. *Nucleic Acids Research*, 42(14): 9195–9208.

Gomez, M., Wong, D., Stewart, T., Redelmeier, D. & Fish, J. 2008. The FLAMES Score Accurately Predicts Mortality Risk in Burn Patients. *The Journal of Trauma*, 65(3): 636–645.

Gomez, R., Murray, C.K., Hospenthal, D.R., Cancio, L.C., Renz, E.M., Holcomb, J.B., Wade, C.E. & Wolf, S.E. 2009. Causes of Mortality by Autopsy Findings of Combat Casualties and Civilian Patients Admitted to a Burn Unit. *Journal of the American College of Surgeons*, 208(3): 348–354.

González-López, P., Álvarez-Villarreal, M., Ruiz-Simón, R., López-Pastor, A.R., de Ceniga, M.V., Esparza, L., Martín-Ventura, J.L., Escribano, Ó. & Gómez-Hernández, A. 2023. Role of miR-15a-5p and miR-199a-3p in the inflammatory pathway regulated by NF-κB in experimental and human atherosclerosis. *Clinical and Translational Medicine*, 13(8): e1363.

Goto, S., Bono, H., Ogata, H., Fujibuchi, W., Nishioka, T., Sato, K. & Kanehisa, M. 1997. Organizing and computing metabolic pathway data in terms of binary relations. *Pacific Symposium on Biocomputing*.175–86.

Gowda, P.S., Zhou, F., Chadwell, L. V. & McEwen, D.G. 2012. p53 Binding Prevents Phosphatase-mediated Inactivation of Diphosphorylated c-Jun N-terminal Kinase. *Journal of Biological Chemistry*, 287(21): 17554–17567.

Graff, J.W., Powers, L.S., Dickson, A.M., Kim, J., Reisetter, A.C., Hassan, I.H., Kremens, K., Gross, T.J., Wilson, M.E. & Monick, M.M. 2012. Cigarette smoking decreases global microRNA expression in human alveolar macrophages. *PLoS One*, 7(8): e44066.

Greenberg, S.S., Ouyang, J., Zhao, X. & Giles, T.D. 1998. Human and rat neutrophils constitutively

express neural nitric oxide synthase mRNA. *Nitric Oxide*, 2(3): 203–212.

Greenhalgh, D.G. 2017. Sepsis in the burn patient: a different problem than sepsis in the general population. *Burns & Trauma*, 5(1): 23.

Gregory, M., Duffner, L., Faunce, D. & Kovacs, E. 2000a. Estrogen mediates the sex difference in post-burn immunosuppression. *Journal of Endocrinology*, 164(2): 129–138.

Gregory, M.S., Faunce, D.E., Duffner, L.A. & Kovacs, E.J. 2000b. Gender difference in cell-mediated immunity after thermal injury is mediated, in part, by elevated levels of interleukin-6. *Journal of Leukocyte Biology*, 67(3): 319–326.

Grivennikov, S.I. & Karin, M. 2010. Dangerous liaisons: STAT3 and NF-κB collaboration and crosstalk in cancer. *Cytokine & Growth Factor Reviews*, 21(1): 11–19.

Gross, A., Jockel, J., Wei, M.C. & Korsmeyer, S.J. 1998. Enforced dimerization of BAX results in its translocation, mitochondrial dysfunction and apoptosis. *The European Molecular Biology Organization Journal*, 17(14): 3878–3885.

Grossman, S.R. 2001. p300/CBP/p53 interaction and regulation of the p53 response. *European Journal of Biochemistry*, 268(10): 2773–2778.

Gruenberg, J. & van der Goot, F. 2006. Mechanism of pathogen entry thorugh the endosomal compartments. *Nature reviews. Molecular Cell Biology*, 7(7): 495–504.

Grunwald, T.B. & Garner, W.L. 2008. Acute burns. *Plastic & Reconstructive Surgery*, 121(5): 311e–319e.

Gudkov, A. V, Gurova, K. V & Komarova, E.A. 2011. Inflammation and p53: A Tale of Two Stresses. *Genes & Cancer*, 2(4): 503–516.

Guery, B., Pittet, J.F. & Marchetti, P. 2002. Apoptosis in Pneumonia. In *Yearbook of Intensive Care and Emergency Medicine 2002*. Berlin, Heidelberg: Springer Berlin Heidelberg: 77–92.

Guinee, D., Fleming, M., Hayashi, T., Woodward, M., Zhang, J., Walls, J., Koss, M., Ferrans, V. & Travis, W. 1996. Association of p53 and WAF1 expression with apoptosis in diffuse alveolar damage. *The American Journal of Pathology*, 149(2): 531–538.

Guo, B., Bai, Y., Ma, Y., Liu, C., Wang, S., Zhao, R., Dong, J. & Ji, H.-L. 2019. Preclinical and clinical studies of smoke-inhalation-induced acute lung injury: update on both pathogenesis and innovative therapy. *Therapeutic Advances in Respiratory Disease*, 13: 1753466619847901–1753466619847901.

Guo, L., Sun, B., Wu, Q., Yang, S. & Chen, F. 2012. MiRNA–miRNA interaction implicates for potential mutual regulatory pattern. *Gene*, 511(2): 187–194.

Guo, Q., Jin, Y., Chen, X., Ye, X., Shen, X., Lin, M., Zeng, C., Zhou, T. & Zhang, J. 2024. NF-κB in biology and targeted therapy: new insights and translational implications. *Signal Transduction & Targeted Therapy*, 9(1): 53.

Gupta, S., Wong, E.G., Mahmood, U., Charles, A.G., Nwomeh, B.C. & Kushner, A.L. 2014. Burn management capacity in low and middle-income countries: A systematic review of 458 hospitals across 14 countries. *International Journal of Surgery*, 12(10): 1070–1073.

Gurzov, E.N., Germano, C.M., Cunha, D.A., Ortis, F., Vanderwinden, J.-M., Marchetti, P., Zhang, L. & Eizirik, D.L. 2010. p53 Up-regulated Modulator of Apoptosis (PUMA) Activation Contributes to Pancreatic β-Cell Apoptosis Induced by Proinflammatory Cytokines and Endoplasmic Reticulum Stress. *Journal of Biological Chemistry*, 285(26): 19910–19920.

Ha, M. & Kim, V.N. 2014. Regulation of microRNA biogenesis. *Nature Reviews Molecular Cell Biology*, 15(8): 509–524.

Haijun, Z., Yonghui, Y., Jiake, C. & Hongjie, D. 2015. Detection of the MicroRNA expression profile in skeletal muscles of burn trauma at the early stage in rats. *Turkish Journal of Trauma & Emergency Surgery*, 21(4): 241–247.

Haik, J., Liran, A., Tessone, A., Givon, A., Orenstein, A., Peleg, K. & Israeli Trauma Group. 2007. Burns in Israel: demographic, etiologic and clinical trends, 1997-2003. *The Israel Medical Association Journal*, 9(9): 659–662.

Haley, R.W., Hooton, T.M., Culver, D.H., Stanley, R.C., Emori, T.G., Hardison, C.D., Quade, D., Shachtman, R.H., Schaberg, D.R., Shah, B. V & Schatz, G.D. 1981. Nosocomial infections in U.S. hospitals, 1975–1976: Estimated frequency by selected characteristics of patients. *The American Journal of Medicine*, 70(4): 947–959.

Hammond, S.M. 2015. An overview of microRNAs. *Advanced Drug Delivery Reviews*, 87: 3–14.

Hampson, N.B. 2008. Stability of carboxyhemoglobin in stored and mailed blood samples. *The American Journal of Emergency Medicine*, 26(2): 191–195.

Han, J., Yoontae, L., Jyu-hyun, Y., Young-kook, K., Hua, J. & Kim, V.. 2004. The Drosha–DGCR8 complex in primary microRNA processing. *Genes & Development*, 18: 3016–3027.

Handschick, K., Beuerlein, K., Jurida, L., Bartkuhn, M., Müller, H., Soelch, J., Weber, A., Dittrich-Breiholz, O., Schneider, H., Scharfe, M., Jarek, M., Stellzig, J., Schmitz, M.L. & Kracht, M. 2014. Cyclin-dependent kinase 6 is a chromatin-bound cofactor for NF-κB-dependent gene expression. *Molecular Cell*, 53(2): 193–208.

Haponik, E.F., Meyers, D.A., Munster, A.M., Smith, P.L., Britt, E.J., Wise, R.A. & Bleecker, E.R. 1987. Acute upper airway injury in burn patients. Serial changes of flow-volume curves and nasopharyngoscopy. *The American Review of Respiratory Disease*, 135(2): 360–366.

Harats, M., Peleg, K., Givon, A., Kornhaber, R., Goder, M., Jaeger, M. & Haik, J. 2016. Burns in Israel, comparative study: Demographic, etiologic and clinical trends 1997–2003 vs. 2004–2010. *Burns*, 42(3): 500–507.

Hardbower, D.M., Singh, K., Asim, M., Verriere, T.G., Olivares-Villagómez, D., Barry, D.P., Allaman, M.M., Washington, M.K., Peek, R.M., Piazuelo, M.B. & Wilson, K.T. 2016. EGFR regulates macrophage activation and function in bacterial infection. *Journal of Clinical Investigation*, 126(9): 3296–3312.

Härkönen, P.L. & Väänänen, H.K. 2006. Monocyte–Macrophage System as a Target for Estrogen and Selective Estrogen Receptor Modulators. *Annals of the New York Academy of Sciences*, 1089(1): 218–227.

Harris, S.L. & Levine, A.J. 2005. The p53 pathway: positive and negative feedback loops. *Oncogene*, 24(17): 2899–2908.

Harvey, S., Martínez-Moreno, C.G., Luna, M. & Arámburo, C. 2015. Autocrine/paracrine roles of extrapituitary growth hormone and prolactin in health and disease: An overview. *General & Comparative Endocrinology*, 220: 103–111.

Hashimoto, T., Ichiki, T., Ikeda, J., Narabayashi, E., Matsuura, H., Miyazaki, R., Inanaga, K., Takeda, K. & Sunagawa, K. 2011. Inhibition of MDM2 attenuates neointimal hyperplasia via suppression of vascular proliferation and inflammation. *Cardiovascular Research*, 91(4): 711–719.

Hassan, Z., Wong, J.K., Bush, J., Bayat, A. & Dunn, K.W. 2010. Assessing the severity of inhalation injuries in adults. *Burns*, 36(2): 212–216.

Häusler, S.F.M., Keller, A., Chandran, P.A., Ziegler, K., Zipp, K., Heuer, S., Krockenberger, M., Engel, J.B., Hönig, A., Scheffler, M., Dietl, J. & Wischhusen, J. 2010. Whole blood-derived miRNA profiles as potential new tools for ovarian cancer screening. *British Journal of Cancer*,

103(5): 693–700.

Hayden, M.S. & Ghosh, S. 2014. Regulation of NF-κB by TNF family cytokines. *Seminars in Immunology*, 26(3): 253–266.

Hayder, H., O'Brien, J., Nadeem, U. & Peng, C. 2018. MicroRNAs: crucial regulators of placental development. *Reproduction*, 155(6): R259–R271.

He, T.-C., Sparks, A.B., Rago, C., Hermeking, H., Zawel, L., da Costa, L.T., Morin, P.J., Vogelstein, B. & Kinzler, K.W. 1998. Identification of c-MYC as a Target of the APC Pathway. *Science*, 281(5382): 1509–1512.

Heid, C.A., Stevens, J.K., Livak, K. & Williams, P. 1996. Real time quantitative PCR. *Genome Research*, 6(10): 986–994.

Heijnen, H.F.G., Schiel, A.E., Fijnheer, R., Geuze, H.J. & Sixma, J.J. 1999. Activated platelets release two types of membrane vesicles: Microvesicles by surface shedding and exosomes derived from exocytosis of multivesicular bodies and alpha-granules. *Blood*, 94(11): 3791–3799.

Hemeda, M., Maher, A. & Mabrouk, A. 2003. Epidemiology of burns admitted to Ain Shams University Burns Unit, Cairo, Egypt. *Burns*, 29(4): 353–358.

Heng, J.S., Clancy, O., Atkins, J., Leon-Villapalos, J., Williams, A.J., Keays, R., Hayes, M., Takata, M., Jones, I. & Vizcaychipi, M.P. 2015. Revised Baux Score and updated Charlson comorbidity index are independently associated with mortality in burns intensive care patients. *Burns*, 41(7): 1420–1427.

Herndon, D.N., Barrow, R.E., Linares, H.A., Rutan, R.L., Prien, T., Traber, L.D. & Traber, D.L. 1988. Inhalation injury in burned patients: effects and treatment. *Burns*, 14(5): 349–356.

Herrero, E., Sánchez, M., Cachafeiro, L., Agrifoglio, A., Galván, B., Asensio, M. & de Lorenzo, A. 2015. Lactate in the burn patient. *Critical Care*, 19(Suppl 1): P145.

Hill, M. & Tran, N. 2022. MiRNA:miRNA Interactions: A Novel Mode of miRNA Regulation and Its Effect On Disease. *Advances in Experimental Medicine & Biology*, 1385: 241–257.

Hill, M. & Tran, N. 2021. MiRNA interplay: Mechanisms and consequences in cancer. *Disease Models & Mechanisms,* 14(4): dmm047662.

Hirano, T., Ishihara, K. & Hibi, M. 2000. Roles of STAT3 in mediating the cell growth, differentiation and survival signals relayed through the IL-6 family of cytokine receptors. *Oncogene*, 19(21): 2548–2556.

Holm, C. 2000. Resuscitation in shock associated with burns. Tradition or evidence-based medicine? *Resuscitation*, 44(3): 157–164.

Hong, B.S., Cho, J.-H., Kim, H., Choi, E.-J., Rho, S., Kim, J., Kim, J.H., Choi, D.-S., Kim, Y.-K., Hwang, D. & Gho, Y.S. 2009. Colorectal cancer cell-derived microvesicles are enriched in cell cycle-related mRNAs that promote proliferation of endothelial cells. *BioMed Central Genomics*, 10(1): 556.

Hong, X., Li, S., Wang, J., Zhao, Z. & Feng, Z. 2021. Circular RNA circFADS2 is overexpressed in sepsis and suppresses LPS-induced lung cell apoptosis by inhibiting the maturation of miR-15a-5p. *BioMed Central Immunology*, 22(1): 29.

Horiuchi, T., Mitoma, H., Harashima, S. -i., Tsukamoto, H. & Shimoda, T. 2010. Transmembrane TNF- : structure, function and interaction with anti-TNF agents. *Rheumatology*, 49(7): 1215–1228.

Hoshyar, R., Bathaie, S.Z. & Sadeghizadeh, M. 2013. Crocin Triggers the Apoptosis Through Increasing the Bax/Bcl-2 Ratio and Caspase Activation in Human Gastric Adenocarcinoma,

AGS, Cells. *DNA & Cell Biology*, 32(2): 50–57.

Hotchkiss, R.S., Moldawer, L.L., Opal, S.M., Reinhart, K., Turnbull, I.R. & Vincent, J.-L. 2016. Sepsis and septic shock. *Nature Reviews Disease Primers*, 2(1): 16045.

Hotchkiss, R.S. & Nicholson, D.W. 2006. Apoptosis and caspases regulate death and inflammation in sepsis. *Nature Reviews Immunology*, 6(11): 813–822.

Hu, Y., Lan, W. & Miller, D. 2017. Next-Generation sequencing for microRNA expression profile. In *Bioinformatics in MicroRNA Research. Methods in Molecular Biology*. New York, NY: Humana Press: 169–177.

Hu, L., Wei, S., Wu, Y., Li, S., Zhu, P. & Wang, X. 2021. MicroRNA regulation of the proliferation and apoptosis of Leydig cells in diabetes. Molecular Medicine, 27(1): 104.

Huang, B.-R., Chen, T.-S., Bau, D.-T., Chuang, I.-C., Tsai, C.-F., Chang, P.-C. & Lu, D.-Y. 2017. EGFR is a pivotal regulator of thrombin-mediated inflammation in primary human nucleus pulposus culture. *Scientific Reports*, 7(1): 8578.

Huang, F., Zhang, J., Yang, D., Zhang, Y., Huang, J., Yuan, Y., Li, X. & Lu, G. 2018. MicroRNA Expression Profile of Whole Blood Is Altered in Adenovirus-Infected Pneumonia Children. *Mediators of Inflammation*, 2018: 2320640.

Huang, H.-Y., Lin, Y.-C.-D., Cui, S., Huang, Y., Tang, Y., Xu, J., Bao, J., Li, Y., Wen, J., Zuo, H., Wang, W., Li, J., Ni, J., Ruan, Y., Li, L., Chen, Yidan, Xie, Y., Zhu, Z., Cai, X., Chen, X., Yao, L., Chen, Yigang, Luo, Y., LuXu, S., Luo, M., Chiu, C.-M., Ma, K., Zhu, L., Cheng, G.-J., Bai, C., Chiang, Y.-C., Wang, L., Wei, F., Lee, T.-Y. & Huang, H.-D. 2022a. miRTarBase update 2022: an informative resource for experimentally validated miRNA–target interactions. *Nucleic Acids Research*, 50(D1): D222–D230.

Huang, J.-Y., Cui, S.-Y., Chen, Y.-T., Song, H.-Z., Huang, G.-C., Feng, B., Sun, M., De, W., Wang, R. & Chen, L.-B. 2013. MicroRNA-650 was a prognostic factor in human lung adenocarcinoma and confers the docetaxel chemoresistance of lung adenocarcinoma cells via regulating Bcl-2/Bax expression. *PLoS One*, 8(8): e72615–e72615.

Huang, R.-Y., Chen, S.-J., Hsiao, Y.-C., Kuo, L.-W., Liao, C.-H., Hsieh, C.-H., Bajani, F. & Fu, C.-Y. 2022b. Positive signs on physical examination are not always indications for endotracheal tube intubation in patients with facial burn. *BioMed Central Emergency Medicine*, 22(1): 36.

Hunt, E.A., Broyles, D., Head, T. & Deo, S.K. 2015. MicroRNA detection: Current technology and research strategies. *Annual Review of Analytical Chemistry*, 8(1): 217–237.

Hunt, J.L., Agee, R.N. & Pruitt Jr, B.A. 1975. Fiberoptic bronchoscopy in acute inhalation injury. *Journal of Trauma*, 15: 641–649.

Hunter, M. & Posel, D. 2012. Here to work: the socioeconomic characteristics of informal dwellers in post-apartheid South Africa. *Environment & Urbanization*, 24(1): 285–304.

Hutvagner, G. & Simard, M.J. 2008. Argonaute proteins: key players in RNA silencing. *Nature Reviews Molecular Cell Biology*, 9(1): 22–32.

Ihling, C., Haendeler, J., Menzel, G., Hess, R.D., Fraedrich, G., Schaefer, H.E. & Zeiher, A.M. 1998. Co-expression of p53 and MDM2 in human atherosclerosis: implications for the regulation of cellularity of atherosclerotic lesions. *The Journal of Pathology*, 185(3): 303–312.

Institute for Health Metrics and Evaluation. 2012. *The Global Burden of Disease 2010 Update*. https://viz.healthmetricsandevaluation.org/gbd-compare/ [12 April 2019].

Ismail, N., Wang, Y., Dakhlallah, D., Moldovan, L., Agarwal, K., Batte, K., Shah, P., Wisler, J., Eubank, T.D., Tridandapani, S., Paulaitis, M.E., Piper, M.G. & Marsh, C.B. 2013. Macrophage

microvesicles induce macrophage differentiation and miR-223 transfer. *Blood*, 121(6): 984–995.

Ivey, K.N. & Srivastava, D. 2015. microRNAs as developmental regulators. *Cold Spring Harbor Perspectives in Biology*, 7(7): a008144–a008144.

Iwasaki, S. & Tomari, Y. 2009. Argonaute-mediated translational repression (and activation). *Fly*, 3(3): 204–206.

Iwaszko, M., Biały, S. & Bogunia-Kubik, K. 2021. Significance of interleukin (IL)-4 and IL-13 in inflammatory arthritis. *Cells*, 10(11): 3000.

Jacob, N.K., Cooley, J. V, Yee, T.N., Jacob, J., Alder, H., Wickramasinghe, P., Maclean, K.H. & Chakravarti, A. 2013. Identification of sensitive serum microRNA biomarkers for radiation biodosimetry. *PLoS One*, 8(2): e57603.

Jafaryparvar, Z., Adib, M., Ghanbari, A. & Ali Yazdanipour, M. 2021. Predictors of short-term outcomes of burn in a newly established burn centre in Iran. *Nursing Open*, 8(6): 2986–2995.

Jagtap, P. & Szabó, C. 2005. Jagtap P, Szabo C.. Poly(ADP-ribose) polymerase and the therapeutic effects of its inhibitors. Nat Rev Drug Discov 4: 421-440. *Nature reviews*, 4(5): 421–440.

Jang, D.-I., Lee, A.-H., Shin, H.-Y., Song, H.-R., Park, J.-H., Kang, T.-B., Lee, S.-R. & Yang, S.-H. 2021. The role of tumor necrosis factor alpha (TNF-α) in autoimmune disease and current TNF-α inhibitors in therapeutics. *International Journal of Molecular Sciences*, 22(5): 2719.

Jansing, J.C., Fiedler, J., Pich, A., Viereck, J., Thum, T., Mühlfeld, C. & Brandenberger, C. 2020. miR-21-KO alleviates alveolar structural remodeling and inflammatory signaling in acute lung injury. *International Journal of Molecular Sciences*, 21(3): 822.

Jasinski-Bergner, S., Mandelboim, O. & Seliger, B. 2014. The Role of MicroRNAs in the control of innate immune response in Ccncer. *Journal of the National Cancer Institute*, 106(10): dju257.

Jazwa, A., Kasper, L., Bak, M., Sobczak, M., Szade, K., Jozkowicz, A., Sladek, K. & Dulak, J. 2015. Differential inflammatory microRNA and cytokine expression in pulmonary sarcoidosis. *Archivum Immunologiae et Therapiae Experimentalis*, 63(2): 139–146.

Jeong, K.S., Zhou, J., Griffin, S.C., Jacobs, E.T., Dearmon-Moore, D., Zhai, J., Littau, S.R., Gulotta, J., Moore, P., Peate, W.F., Richt, C.M. & Burgess, J.L. 2018. MicroRNA changes in firefighters. *Journal of Occupational & Environmental Medicine*, 60(5): 469–474.

Jeschke, M.G., Mlcak, R.P., Finnerty, C.C., Norbury, W.B., Przkora, R., Kulp, G.A., Gauglitz, G.G., Zhang, X.-J. & Herndon, D.N. 2008. Gender differences in pediatric burn patients. *Annals of Surgery*, 248(1): 126–136.

Jeschke, M.G., Patsouris, D., Stanojcic, M., Abdullahi, A., Rehou, S., Pinto, R., Chen, P., Burnett, M. & Amini-Nik, S. 2015. Pathophysiologic response to burns in the elderly. *EBioMedicine*, 2(10): 1536–1548.

Jeschke, M.G., van Baar, M.E., Choudhry, M.A., Chung, K.K., Gibran, N.S. & Logsetty, S. 2020. Burn injury. *Nature Reviews*, 6(1): 11.

Jia, Z., Wang, K., Wang, G., Zhang, A. & Pu, P. 2013. Correction: MiR-30a-5p antisense oligonucleotide suppresses glioma cell growth by targeting SEPT7. *PLoS One*, 8(10): 10.

Jiang, H., Zhao, P.-J., Su, D., Fend, J. & Ma, S.-L. 2014. Paris saponin I induces apoptosis via increasing the Bax/Bcl-2 ratio and caspase-3 expression in gefitinib-resistant non-small cell lung cancer in vitro and in vivo. *Molecular Medicine Reports*, 9(6): 2265–2272.

Jiang, W., Ren, J., Zhang, X., Lu, Z., Feng, H., Yao, X., Li, D., Xiong, R., Fan, T. & Geng, Q. 2020. CircC3P1 attenuated pro-inflammatory cytokine production and cell apoptosis in acute lung injury induced by sepsis through modulating miR-21. *Journal of Cellular & Molecular Medicine*,

24(19): 11221–11229.

Jiang, Z.-Y., Liu, M.-Z., Fu, Z.-H., Liao, X.-C., Xu, B., Shi, L.-L., Li, J.-Q. & Guo, G.-H. 2022. The expression profile of lung long non-coding RNAs and mRNAs in a mouse model of smoke inhalation injury. *Bioengineered*, 13(3): 4978–4990.

Jiburum, B. & Olaitan, P. 2005. Burn injuries in Enugu, Nigeria. *Nigerian Journal of Surgical Research*, 7(3): 271–273.

Jie, X., Haijun, W., Zhiqiang, W., Guoyou, F. & Guanghui, H. 2003. Correlation between standardized death rate for area and LA50. *Burns*, 29(3): 257–264.

Joh, E.-H., Gu, W. & Kim, D.-H. 2012. Echinocystic acid ameliorates lung inflammation in mice and alveolar macrophages by inhibiting the binding of LPS to TLR4 in NF-κB and MAPK pathways. *Biochemical Pharmacology*, 84(3): 331–340.

Johnnidis, J.B., Harris, M.H., Wheeler, R.T., Stehling-Sun, S., Lam, M.H., Kirak, O., Brummelkamp, T.R., Fleming, M.D. & Camargo, F.D. 2008. Regulation of progenitor cell proliferation and granulocyte function by microRNA-223. *Nature*, 451(7182): 1125–1129.

Johnson, C.D., Esquela-Kerscher, A., Stefani, G., Byrom, M., Kelnar, K., Ovcharenko, D., Wilson, M., Wang, X., Shelton, J., Shingara, J., Chin, L., Brown, D. & Slack, F.J. 2007. The let-7 microRNA represses cell proliferation pathways in human cells. *Cancer Research*, 67(16): 7713 – 7722.

Jones, S.W., Williams, F.N., Cairns, B.A. & Cartotto, R. 2017. Inhalation injury: Pathophysiology, diagnosis, and treatment. *Clinics in Plastic Surgery*, 44(3): 505–511.

Jones, S.W., Zhou, H., Ortiz-Pujols, S.M., Maile, R., Herbst, M., Joyner Jr, B.L., Zhang, H., Kesic, M., Jaspers, I., Short, K.A., Meyer, A.A., Peden, D.B., Cairns, B.A. & Noah, T.L. 2013. Bronchoscopy-derived correlates of lung injury following inhalational injuries: a prospective observational study. *PLoS One*, 8(5): e64250–e64250.

Jordan, K.C., Di Gennaro, J.L., von Saint André-von Arnim, A. & Stewart, B.T. 2022. Global trends in pediatric burn injuries and care capacity from the World Health Organization Global Burn Registry. *Frontiers in Pediatrics*, 10: 954995.

Joshi-Tope, G. 2005. Reactome: a knowledgebase of biological pathways. *Nucleic Acids Research*, 33: D428–D432.

Joshi, V.D., Kalvakolanu, D. V & Cross, A.S. 2003. Simultaneous activation of apoptosis and inflammation in pathogenesis of septic shock: a hypothesis 1. *FEBS Letters*, 555(2): 180–184.

Jubran, A. & Tobin, M.J. 2013. Monitoring during mechanical ventilation. In Tobin, M.J. (ed.) *Principles & Practice of Mechanical Ventilation*. New York: McGraw-Hill Inc: 261–287.

Jugmohan, B., Loveland, J., Doedens, L., Moore, R.L., Welthagen, A. & Westgarth-Taylor, C.J. 2016. Mortality in paediatric burns victims: A retrospective review from 2009 to 2012 in a single centre. *South African Medical Journal*, 106(2): 189.

Juncadella, I.J., Kadl, A., Sharma, A.K., Shim, Y.M., Hochreiter-Hufford, A., Borish, L. & Ravichandran, K.S. 2013. Apoptotic cell clearance by bronchial epithelial cells critically influences airway inflammation. *Nature*, 493(7433): 547–551.

Junttila, I.S. 2018. Tuning the Cytokine Responses: An update on interleukin (IL)-4 and IL-13 receptor complexes. *Frontiers in Immunology*, 9: 888.

Kaita, Y., Tarui, T., Tanaka, Y., Suzuki, J., Yoshikawa, K. & Yamaguchi, Y. 2020. Reevaluation for prognostic value of prognostic burn index in severe burn patients. *Acute Medicine & Surgery*, 7(1): e499–e499.

Kalayi, G.D. 2001. Epidemiology of burn injury: Experience in the Savannah region of Northern Nigeria. *Tropical Doctor*, 31(4): 209–211.

Kamolz, L.-P., Andel, H., Schramm, W., Meissl, G., Herndon, D.N. & Frey, M. 2005. Lactate: early predictor of morbidity and mortality in patients with severe burns. *Burns,* 31(8): 986–990.

Kanehisa, M., Furumichi, M., Tanabe, M., Sato, Y. & Morishima, K. 2017. KEGG: new perspectives on genomes, pathways, diseases and drugs. *Nucleic Acids Research*, 45(D1): D353–D361.

Kanzi, A.M., San, J.E., Chimukangara, B., Wilkinson, E., Fish, M., Ramsuran, V. & de Oliveira, T. 2020. Next generation sequencing and bioinformatics analysis of family genetic inheritance. *Frontiers in Genetics*, 11: 544162.

Karami, M.B., Karami, M.R., Ahmadi, J.T., Ghahvehei, N., Haghi, M., Ahmadi, M. & Rezaei, S. 2012. Epidemiological data, outcome, and costs of burn patients in Kermanshah. *Annals of Burns & Fire Disasters*, 25(4): 171–177.

Karimi, H., Motevalian, A., Momeni, M. & Vasigh, M. 2016. Epidemiology and outcome of inhalation injury due to flame burn injuries in Iran. *European Journal of Pharmaceutical & Medical Research*, 3(5): 319–324.

Karimi, H., Motevalian, S.-A., Rabbani, A., Motabar, A.-R., Vasigh, M., Sabzeparvar, M. & Mobayen, M. 2013. Prediction of mortality in pediatric burn injuries: R-baux score to be applied in children (pediatrics-baux score). *Iranian Journal of Pediatrics*, 23(2): 165–170.

Karimi, K., Faraklas, I., Lewis, G., Ha, D., Walker, B., Zhai, Y., Graves, G. & Dissanaike, S. 2017. Increased mortality in women: Sex differences in burn outcomes. *Burns & Trauma*, 5: 18.

Karolina, D.S., Armugam, A., Tavintharan, S., Wong, M.T.K., Lim, S.C., Sum, C.F. & Jeyaseelan, K. 2011. MicroRNA 144 impairs insulin signaling by inhibiting the expression of insulin receptor substrate 1 in type 2 diabetes mellitus. *PLoS One*, 6(8): e22839.

Karpelowsky, J.S., Wallis, L., Madaree, A. & Rode, H. 2007. South African Burn Society burn stabilisation protocol. *South African Medical Journal*, 97(8): 574.

Kasten, K.R., Makley, A.T. & Kagan, R.J. 2011. Update on the critical care management of severe burns. *Journal of Intensive Care Medicine*, 26(4): 223–236.

Kawasaki, M., Kuwano, K., Hagimoto, N., Matsuba, T., Kunitake, R., Tanaka, T., Maeyama, T. & Hara, N. 2000. Protection from lethal apoptosis in lipopolysaccharide-induced acute lung injury in mice by a caspase inhibitor. *The American Journal of Pathology*, 157(2): 597–603.

Kay, J., Thadhani, E., Samson, L. & Engelward, B. 2019. Inflammation-induced DNA damage, mutations and cancer. *DNA Repair*, 83: 102673.

Kealey, G.P. 2009. Carbon monoxide toxicity. *Journal of Burn Care & Research*, 30(1): 146–147.

Keen III, E.F., Robinson, B.J., Hospenthal, D.R., Aldous, W.K., Wolf, S.E., Chung, K.K. & Murray, C.K. 2010. Incidence and bacteriology of burn infections at a military burn center. *Burns*, 36(4): 461–468.

Keller, A., Leidinger, P., Bauer, A., ElSharawy, A., Haas, J., Backes, C., Wendschlag, A., Giese, N., Tjaden, C., Ott, K., Werner, J., Hackert, T., Ruprecht, K., Huwer, H., Huebers, J., Jacobs, G., Rosenstiel, P., Dommisch, H., Schaefer, A., Müller-Quernheim, J., Wullich, B., Keck, B., Graf, N., Reichrath, J., Vogel, B., Nebel, A., Jager, S.U., Staehler, P., Amarantos, I., Boisguerin, V., Staehler, C., Beier, M., Scheffler, M., Büchler, M.W., Wischhusen, J., Haeusler, S.F.M., Dietl, J., Hofmann, S., Lenhof, H.-P., Schreiber, S., Katus, H.A., Rottbauer, W., Meder, B., Hoheisel, J.D., Franke, A. & Meese, E. 2011. Toward the blood-borne miRNome of human diseases. *Nature Methods*, 8(10): 841–843.

Kennedy, S. & Cahill, K.C. 2020. Cyanide poisoning in inhalation injuries. *Clinical Case Reports*, 8(12): 3566–3567.

Kerby, J.D., McGwin, G., George, R.L., Cross, J.A., Chaudry, I.H. & Rue, L.W. 2006. Sex differences in mortality after burn injury: Results of analysis of the national burn repository of the American Burn Association. *Journal of Burn Care & Research*, 27(4): 452–456.

Kern-Lunbery, R.J., Rathert-Williams, A.R., Foote, A.P., Cunningham-Hollinger, H.C., Kuehn, L.A., Meyer, A.M. & Lindholm-Perry, A.K. 2024. Genes involved in the cholecystokinin receptor signaling map were differentially expressed in the jejunum of steers with variation in residual feed intake. *Veterinary & Animal Science*, 24: 100357.

Keyes, C. & Liphoko, K. 2021. A five-year overview of fatal thermal and electrical burns in Johannesburg, South Africa. *South African Journal of Science*, 117(5–6): 1–9.

Kida, Y., Kobayashi, M., Suzuki, T., Takeshita, A., Okamatsu, Y., Hanazawa, S., Yasui, T. & Hasegawa, K. 2005. Interleukin-1 stimulates cytokines, prostaglandin E2 and matrix metalloproteinase-1 production via activation of MAPK/AP-1 and NF-κB in human gingival fibroblasts. *Cytokine*, 29(4): 159–168.

Kim, J., Lee, J.N., Ye, James, Hao, R., DeBose-Boyd, R. & Ye, Jin. 2013. Sufficient production of geranylgeraniol is required to maintain endotoxin tolerance in macrophages. *Journal of Lipid Research*, 54(12): 3430–3437.

Kim, J.-E. & Yang, S.-J. 2022. miR-30a-5p augments the anti-inflammatory effects of dexmedetomidine in LPS-induced BV2 cells. *Korean Journal of Clinical Laboratory Science*, 54(3): 201–208.

Kim, R.Y., Sunkara, K.P., Bracke, K.R., Jarnicki, A.G., Donovan, C., Hsu, A.C., Ieni, A., Beckett, E.L., Galvão, I., Wijnant, S., Ricciardolo, F.L., Di Stefano, A., Haw, T.J., Liu, G., Ferguson, A.L., Palendira, U., Wark, P.A., Conickx, G., Mestdagh, P., Brusselle, G.G., Caramori, G., Foster, P.S., Horvat, J.C. & Hansbro, P.M. 2021. A microRNA-21–mediated SATB1/S100A9/NF-κB axis promotes chronic obstructive pulmonary disease pathogenesis. *Science Translational Medicine*, 13(621): eaav7223.

Kim, Y., Kym, D., Hur, J., Jeon, J., Yoon, J., Yim, H., Cho, Y.S. & Chun, W. 2019. Development of a risk prediction model (Hangang) and comparison with clinical severity scores in burn patients. *PLoS One*, 14(2): e0211075–e0211075.

Kim, Y., Kym, D., Hur, J., Yoon, J., Yim, H. & Cho, Y.S. 2017. Does inhalation injury predict mortality in burns patients or require redefinition? *PLoS One*, 12(9): e0185195.

Kimemia, D., Vermaak, C., Pachauri, S. & Rhodes, B. 2014. Burns, scalds and poisonings from household energy use in South Africa: Are the energy poor at greater risk? *Energy for Sustainable Development*, 18: 1–8.

Kimmel, E.C. & Still, K.R. 1999. Acute lung injury, acute respiratory distress syndrome and inhalation injury: an overview. *Drug & Chemical Toxicology*, 22(1): 91–128.

Kiragu, A.W., Dunlop, S.J., Mwarumba, N., Gidado, S., Adesina, A., Mwachiro, M., Gbadero, D.A. & Slusher, T.M. 2018. Pediatric trauma care in low resource settings: Challenges, opportunities, and solutions. *Frontiers in Pediatrics*, 6: 155.

Klein, D., Einspanier, R., Bolder, U. & Jeschke, M.G. 2003. Differences in the hepatic signal transcription pathway and cytokine expression between thermal injury and sepsis. *Shock*, 20(6): 536–543.

Klemsz, M.J., Justement, L.B., Palmer, E. & Cambier, J.C. 1989. Induction of c-fos and c-myc expression during B cell activation by IL-4 and immunoglobulin binding ligands. *Journal of Immunology*, 143(3): 1032–1039.

Kluck, R.M., Bossy-Wetzel, E., Green, D.R. & Newmeyer, D.D. 1997. The release of cytochrome c from mitochondria: A primary site for Bcl-2 regulation of apoptosis. *Science*, 275(5303): 1132–1136.

Knowlin, L., Stanford, L., Moore, D., Cairns, B. & Charles, A. 2016. The measured effect magnitude of co-morbidities on burn injury mortality. *Burns*, 42(7): 1433–1438.

Koh, E.-H., Chernis, N., Saha, P.K., Xiao, L., Bader, D.A., Zhu, B., Rajapakshe, K., Hamilton, M.P., Liu, X., Perera, D., Chen, X., York, B., Trauner, M., Coarfa, C., Bajaj, M., Moore, D.D., Deng, T., McGuire, S.E. & Hartig, S.M. 2018. MiR-30a remodels subcutaneous adipose tissue inflammation to improve insulin sensitivity in obesity. *Diabetes*, 67(12): 2541–2553.

Kong, Y. & Han, J.H. 2005. MicroRNA: Biological and computational perspective. *Genomics, Proteomics & Bioinformatics*, 3(2): 62–72.

Kozomara, A. & Griffiths-Jones, S. 2011. MiRBase: Integrating microRNA annotation and deep-sequencing data. *Nucleic Acids Research*, 39: D152–D157.

Kramer, G.C., Herndon, D.N., Linares, H.A. & Traber, D.L. 1989. Effects of inhalation injury on airway blood flow and edema formation. *The Journal of Burn Care & Rehabilitation*, 10(1): 45–51.

Kraut, J.A. & Kurtz, I. 2006. Use of base in the treatment of acute severe organic acidosis by nephrologists and critical care physicians: results of an online survey. *Clinical & Experimental Nephrology*, 10(2): 111–117.

Kraut, J.A. & Madias, N.E. 2014. Lactic acidosis. *New England Journal of Medicine*, 371(24): 2309–2319.

Krishnan, P., Frew, Q., Green, A., Martin, R. & Dziewulski, P. 2013. Cause of death and correlation with autopsy findings in burns patients. *Burns*, 39(4): 583–588.

Kroh, E.M., Parkin, R.K., Mitchell, P.S. & Tewari, M. 2010. Analysis of circulating microRNA biomarkers in plasma and serum using quantitative reverse transcription-PCR (qRT-PCR). *Methods*, 50(4): 298–301.

Kubo, H., Hayashi, T., Ago, K., Ago, M., Kanekura, T. & Ogata, M. 2014. Temporal expression of wound healing-related genes in skin burn injury. *Legal Medicine*, 16(1): 8–13.

Kuleshov, M. V., Jones, M.R., Rouillard, A.D., Fernandez, N.F., Duan, Q., Wang, Z., Koplev, S., Jenkins, S.L., Jagodnik, K.M., Lachmann, A., McDermott, M.G., Monteiro, C.D., Gundersen, G.W. & Ma'ayan, A. 2016. Enrichr: a comprehensive gene set enrichment analysis web server 2016 update. *Nucleic Acids Research*, 44(W1): W90–W97.

Kwack, W., Lee, D.-S., Min, H., Choi, Y.Y., Yun, M., Kim, Y., Song, I.-A., Park, J.S., Cho, Y.-J., Jo, Y., Yoon, H., Lee, J., Lee, C.-T. & Lee, Y. 2018. Evaluation of the SpO2/FiO2 ratio as a predictor of intensive care unit transfers in respiratory ward patients for whom the rapid response system has been activated. *PLoS One*, 13(7): e0201632.

Lachiewicz, A.M., Hauck, C.G., Weber, D.J., Cairns, B.A. & van Duin, D. 2017. Bacterial infections after burn injuries: Impact of multidrug resistance. *Clinical Infectious Diseases*, 65(12): 2130–2136.

Lagos-Quintana, M., Rauhut, R., Lendeckel, W. & Tuschl, T. 2001. Identification of novel genes coding for small expressed RNAs. *Science*, 294(5543): 853–858.

Lai, E.C. 2002. Micro RNAs are complementary to 3'UTR sequence motifs that mediate negative post-transcriptional regulation. *Nature Genetics*, 30(4): 363–364.

Lai, E.C., Wiel, C. & Rubin, G.M. 2004. Complementary miRNA pairs suggest a regulatory role for

miRNA:miRNA duplexes. RNA, 10(2): 171–175.

Laliberte, A.M., Karadimas, S.K., Vidal, P.M., Satkunendrarajah, K. & Fehlings, M.G. 2021. Mir21 modulates inflammation and sensorimotor deficits in cervical myelopathy: Data from humans and animal models. Brain Communications, 3(1): fcaa234.

Laloë, V. 2002. Epidemiology and mortality of burns in a general hospital of Eastern Sri Lanka. *Burns*, 28(8): 778–781.

Lam, H.Y.K., Clark, M.J., Chen, Rui, Chen, Rong, Natsoulis, G., O'Huallachain, M., Dewey, F.E., Habegger, L., Ashley, E.A., Gerstein, M.B., Butte, A.J., Ji, H.P. & Snyder, M. 2011. Performance comparison of whole-genome sequencing platforms. *Nature Biotechnology*, 30(1): 78–82.

Lam, N.N. & Hung, T.D. 2019. ARDS among cutaneous burn patients combined with inhalation injury: Early onset and bad outcome. *Annals of Burns & Fire Disasters*, 32(1): 37–42.

Lamhamedi-Cherradi, S.-E., Zheng, S.-J., Maguschak, K.A., Peschon, J. & Chen, Y.H. 2003. Defective thymocyte apoptosis and accelerated autoimmune diseases in TRAIL−/− mice. *Nature Immunology*, 4(3): 255–260.

Lange, M., Connelly, R., Traber, D., Hamahata, A., Nakano, Y., Esechie, A., Jonkam, C., Borzyskowski, S., Traber, L., Schmalstieg, F., Herndon, D. & Enkhbaatar, P. 2010. Time course of nitric oxide synthases, nitrosative stress, and poly(ADP ribosylation) in an ovine sepsis model. *Critical Care*, 14(4): R129.

Lange, M., Enkhbaatar, P., Traber, D.L., Cox, R.A., Jacob, S., Mathew, B.P., Hamahata, A., Traber, L.D., Herndon, D.N. & Hawkins, H.K. 2009. Role of calcitonin gene-related peptide (CGRP) in ovine burn and smoke inhalation injury. *Journal of Applied Physiology*, 107(1): 176–184.

Lange, M., Szabó, C., Traber, D., Horváth, E., Hamahata, A., Nakano, Y., Traber, L., Cox, R., Schmalstieg, F., Herndon, D. & Enkhbaatar, P. 2012. Time profile of oxidative stress and neutrophil activation in ovine acute lung injury and sepsis. *Shock*, 37(5): 468–472.

Latenser, B.A., Miller, S.F., Bessey, P.Q., Browning, S.M., Caruso, D.M., Gomez, M., Jeng, J.C., Krichbaum, J.A., Lentz, C.W., Saffle, J.R., Schurr, M.J., Greenhalgh, D.G. & Kagan, R.J. 2007. National Burn Repository 2006: A Ten-Year Review. *Journal of Burn Care & Research*, 28(5): 635–658.

Laterza, O.F., Lim, L., Garrett-Engele, P.W., Vlasakova, K., Muniappa, N., Tanaka, W.K., Johnson, J.M., Sina, J.F., Fare, T.L., Sistare, F.D. & Glaab, W.E. 2009. Plasma microRNAs as sensitive and specific biomarkers of tissue injury. *Clinical Chemistry*, 55(11): 1977–1983.

Latorre, I., Leidinger, P., Backes, C., Domínguez, J., de Souza-Galvão, M.L., Maldonado, J., Prat, C., Ruiz-Manzano, J., Sánchez, F., Casas, I., Keller, A., von Briesen, H., Knobel, H., Meese, E. & Meyerhans, A. 2015. A novel whole-blood miRNA signature for a rapid diagnosis of pulmonary tuberculosis. *European Respiratory Journal*, 45(4): 1173–1176.

Laycock, H. & Rajah, A. 2010. Acute lung injury and acute respiratory distress syndrome : A review article. *British Journal of Medical Practitioners*, 3(2): 324.

Le, S., Pellegrini, E., Green-Saxena, A., Summers, C., Hoffman, J., Calvert, J. & Das, R. 2020. Supervised machine learning for the early prediction of acute respiratory distress syndrome (ARDS). *Journal of Critical Care*, 60: 96–102.

LeBlanc, V. & Cox, M. 2017. Interpretation of the point-biserial correlation coefficient in the context of a school examination. *The Quantitative Methods for Psychology*, 13: 46–56.

Lee-Chiong Jr, T.L. 1999. Smoke inhalation injury—when to suspect and how to treat. *Postgraduate Medicine*, 105: 55–62.

Lee, C.-H., Hung, H.-W., Hung, P.-H. & Shieh, Y.-S. 2010. Epidermal growth factor receptor regulates β-catenin location, stability, and transcriptional activity in oral cancer. *Molecular Cancer*, 9(1): 64.

Lee, R.C., Feinbaum, R.L. & Ambros, V. 1993. Lee Feinbaum Ambros the C Elegans heterochronic gene lin4 encodes small RNAs with antisense complementarity to lin14. *Cell*, 75: 843–854.

Lee, R.E.C., Walker, S.R., Savery, K., Frank, D.A. & Gaudet, S. 2014. Fold Change of Nuclear NF-κB Determines TNF-Induced Transcription in Single Cells. *Molecular Cell*, 53(6): 867–879.

Lefort, K., Mandinova, A., Ostano, P., Kolev, V., Calpini, V., Kolfschoten, I., Devgan, V., Lieb, J., Raffoul, W., Hohl, D., Neel, V., Garlick, J., Chiorino, G. & Dotto, G.P. 2007. Notch1 is a p53 target gene involved in human keratinocyte tumor suppression through negative regulation of ROCK1/2 and MRCKα kinases. *Genes & Development*, 21(5): 562–577.

Leidinger, P., Backes, C., Dahmke, I.N., Galata, V., Huwer, H., Stehle, I., Bals, R., Keller, A. & Meese, E. 2014. What makes a blood cell based miRNA expression pattern disease specific? A miRNome analysis of blood cell subsets in lung cancer patients and healthy controls. *Oncotarget*, 5(19): 9484–9497.

Levens, D. 2010. 'You Don't Muck with MYC'. *Genes & Cancer*, 1(6): 547–554.

Levite, M. 1998. Neuropeptides, by direct interaction with T cells, induce cytokine secretion and break the commitment to a distinct T helper phenotype. *Proceedings of the National Academy of Sciences of the United States of America*, 95(21): 12544–12549.

Lewis, B.P., Burge, C.B. & Bartel, D.P. 2005. Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets. *Cell*, 120(1): 15–20.

Li, B., Martin, E. & Morris, J. 2001. Latent variable selection in partial least squares modelling. *IFAC Proceedings Volumes*, 34(25): 463–468.

Li, C., Li, Y., Bai, L., He, C. & Yu, X. 2016a. Dynamic expression of miRNAs and their targets in the response to drought stress of grafted cucumber seedlings. *Horticultural Plant Journal*, 2(1): 41–49.

Li, J.-X., Li, Y., Xia, T. & Rong, F.-Y. 2021a. MiR-21 exerts anti-proliferative and pro-apoptotic effects in LPS-induced WI-38 cells via directly targeting TIMP3. *Cell Biochemistry & Biophysics*, 79(4): 781–790.

Li, J., Batcha, A.M.N., Grüning, B. & Mansmann, U.R. 2015. An NGS workflow blueprint for DNA sequencing data and its application in individualized molecular incology. *Cancer Informatics*, 14(Suppl 5): 87–107.

Li, J., Zhou, D., Qiu, W., Shi, Y., Yang, J.-J., Chen, S., Wang, Q. & Pan, H. 2018. Application of weighted gene co-expression network analysis for data from paired design. *Scientific Reports*, 8(1): 622.

Li, L.-F., Ouyang, B., Choukroun, G., Matyal, R., Mascarenhas, M., Jafari, B., Bonventre, J., Force, T. & Quinn, D. 2003. Stretch-induced IL-8 depends on c-Jun NH 2 -terminal and nuclear factor-κB-inducing kinases. *American Journal of Physiology*, 285(2): L464-475.

Li, N., Pan, J., Liu, W., Li, Y., Li, F. & Liu, M. 2020. MicroRNA-15a-5p serves as a potential biomarker and regulates the viability and apoptosis of hippocampus neuron in children with temporal lobe epilepsy. *Diagnostic Pathology*, 15(1): 46.

Li, P., He, Q. & Luo, C. 2014. Overexpression of miR-200b inhibits the cell proliferation and promotes apoptosis of human hypertrophic scar fibroblasts in vitro. *The Journal of Dermatology*, 41(10): 903–911.

Li, P., Nijhawan, D., Budihardjo, I., Srinivasula, S.M., Ahmad, M., Alnemri, E.S. & Wang, X. 1997. Cytochrome c and dATP-dependent formation of Apaf-1/Caspase-9 complex initiates an apoptotic protease cascade. *Cell*, 91(4): 479–489.

Li, P., Yao, Y., Ma, Y. & Chen, Y. 2021b. MiR-30a-5p ameliorates LPS-induced inflammatory injury in human A549 cells and mice via targeting RUNX2. *Innate Immunity*, 27(1): 41–49.

Li, W., Dai, H., Ou, Q., Zuo, G. & Liu, C. 2016b. Overexpression of microRNA-30a-5p inhibits liver cancer cell proliferation and induces apoptosis by targeting MTDH/PTEN/AKT pathway. *Tumor Biology*, 37(5): 5885–5895.

Li, W., Fang, Q., Zhong, P., Chen, L., Wang, L., Zhang, Y., Wang, Jun, Li, X., Wang, Y., Wang, Jingying & Liang, G. 2016c. EGFR Inhibition blocks palmitic acid-induced inflammation in cardiomyocytes and prevents hyperlipidemia-induced cardiac injury in mice. *Scientific Reports*, 6(1): 24580.

Li, X., Shu, R., Filippatos, G. & Uhal, B.D. 2004. Apoptosis in lung injury and remodeling. *Journal of Applied Physiology*, 97(4): 1535–1542.

Li, X., Zhang, X., Zhang, H. & Yu, X. 2024a. MiR 15a 5p inhibits angiogenesis and suppresses inflammation and fibrosis via targeting VEGF and smad2 in ocular neovascularization. *Investigative Ophthalmology & Visual Science*, 65(7): OD62–OD62.

Li, Y., Zhao, X., Liu, Z., Lu, C., Wang, Y., Liu, J., Mou, Y., Zhang, Y., Li, B. & Song, X. 2024b. MiR-10b-5p alleviates Th2 inflammatory response by targeting SHP2 phosphatase in allergic rhinitis. *Allergy*, 00: 1–4.

Li, Z.-W., Zhao, J.-J., Li, S.-Y., Cao, T.-T., Wang, Y., Guo, Y. & Xi, G.-J. 2021c. Blocking the EGFR/p38/NF-κB signaling pathway alleviates disruption of BSCB and subsequent inflammation after spinal cord injury. *Neurochemistry International*, 150: 105190.

Liang, G., Guo, C., Tang, H. & Zhang, M. 2024. MiR-30a-5p attenuates hypoxia/reoxygenation-induced cardiomyocyte apoptosis by regulating PTEN protein expression and activating PI3K/Akt signaling pathway. *BioMed Central Cardiovascular Disorders*, 24(1): 236.

Liang, P., Lv, C., Jiang, B., Long, X., Zhang, P., Zhang, M., Xie, T. & Huang, X. 2012. MicroRNA profiling in denatured dermis of deep burn patients. *Burns*, 38(4): 534–540.

Liao, J., Liu, R., Yin, L. & Pu, Y. 2014. Expression profiling of exosomal miRNAs derived from human esophageal cancer cells by Solexa High-Throughput Sequencing. *International Journal of Molecular Sciences*, 15(9): 15530–15551.

Liaudet, L., Pacher, P., Mabley, J., Virág, L., Soriano, F., Haskó, G. & Szabó, C. 2002. Activation of poly(ADP-Ribose) polymerase-1 is a central mechanism of lipopolysaccharide-induced acute lung inflammation. *American Journal of Respiratory & Critical Care Medicine*, 165(3): 372–377.

Liffner, G., Bak, Z., Reske, A. & Sjöberg, F. 2005. Inhalation injury assessed by score does not contribute to the development of acute respiratory distress syndrome in burn victims. *Burns*, 31(3): 263–268.

Lin, J., Wei, Q. & Fang, Z. 2025. CircRBM33 competitively binds miR-15a-5p to mediate EZH1 expression to ameliorate sepsis-induced acute lung injury. *Clinics*, 80: 100550.

Lin, W., Zhou, L., Liu, M., Zhang, D., Yan, Y., Chang, Y.-F., Zhang, X., Xie, Q. & Luo, Q. 2020. gga-miR-200b-3p promotes macrophage activation and differentiation via targeting monocyte to macrophage differentiation-associated in HD11 cells. *Frontiers in Immunology*, 11: 563143.

Lindner, G. & Exadaktylos, A. 2013. How noninvasive haemoglobin measurement with pulse CO-oximetry can change your practice: An expert review. *Emergency Medicine International*, 2013:

701529.

Liodaki, E., Kalousis, K., Mauss, K.L., Kisch, T., Mailaender, P. & Stang, F. 2015. Epidemiology of pneumonia in a burn care unit: the influence of inhalation trauma on pneumonia and of pneumonia on burn mortality. *Annals of Burns & Fire Disasters*, 28(2): 128–33.

Lipovy, B., Rihová, H., Gregorova, N., Hanslianova, M., Zaloudikova, Z., Kaloudova, Y. & Brychta, P. 2011. Epidemiology of ventilator-associated tracheobronchitis and vantilator-associated pneumonia in patients with inhalation injury at the Burn Centre in Brno (Czech Republic). *Annals of Burns & Fire Disasters*, 24(3): 120–125.

Liu, A., Tetzlaff, M.T., Vanbelle, P., Elder, D., Feldman, M., Tobias, J.W., Sepulveda, A.R. & Xu, X. 2009a. MicroRNA expression profiling outperforms mRNA expression profiling in formalin-fixed paraffin-embedded tissues. *International Journal of Clinical & Experimental Pathology*, 2(6): 519–527.

Liu, E., Sun, X., Li, J. & Zhang, C. 2018. miR-30a-5p inhibits the proliferation, migration and invasion of melanoma cells by targeting SOX4. *Molecular Medicine Reports*, 18(2): 2492–2498.

Liu, G., Park, Y.-J., Tsuruta, Y., Lorne, E. & Abraham, E. 2009b. p53 attenuates lipopolysaccharide-induced NF-κB activation and acute lung injury. *The Journal of Immunology*, 182(8): 5063–5071.

Liu, J., Jennings, S.F., Tong, W. & Hong, H. 2011. Next generation sequencing for profiling expression of miRNAs: Technical progress and applications in drug development. *Journal of Biomedical Science & Engineering*, 4(10): 666–676.

Liu, L., Li, Y., Li, S., Hu, N., He, Y., Pong, R., Lin, D., Lu, L. & Law, M. 2012. Comparison of Next-Generation Sequencing Systems. *Journal of Biomedicine and Biotechnology*, 2012: 251364.

Liu, L., Lu, Y., Martinez, J., Bi, Y., Lian, G., Wang, T., Milasta, S., Wang, J., Yang, M., Liu, G., Green, D.R. & Wang, R. 2016. Proinflammatory signal suppresses proliferation and shifts macrophage metabolism from Myc-dependent to HIF1 α -dependent. *Proceedings of the National Academy of Sciences*, 113(6): 1564–1569.

Liu, R., Du, J., Zhou, J., Zhong, B., Ba, L., Zhang, J., Liu, Y. & Liu, S. 2021a. Elevated microRNA-21 Is a brake of inflammation involved in the development of nasal polyps. *Frontiers in Immunology*, 12: 530488.

Liu, S., Liu, H., Gong, C., Li, G., Li, Q., Pan, Z., He, X., Jiang, Z., Li, H. & Zhang, C. 2024. MiR-10b-5p regulates neuronal autophagy and apoptosis induced by spinal cord injury through UBR7. *Neuroscience,* 543: 13–27.

Liu, T., Zhang, L., Joo, D. & Sun, S.-C. 2017. NF-κB signaling in inflammation. *Signal Transduction & Targeted Therapy*, 2(1): 17023.

Liu, T., Zhou, Y., Ko, K.S. & Yang, H. 2015. Interactions between Myc and mediators of inflammation in chronic liver diseases. *Mediators of Inflammation*, 2015: 276850.

Liu, X., Chen, F., Tan, F., Li, F., Yi, R., Yang, D. & Zhao, X. 2020. Construction of a potential breast cancer-related miRNA-mRNA regulatory network. *BioMed Research International*, 2020: 1–18.

Liu, X, Yang, B., Zhang, Y., Guo, X., Yang, Q., Liu, Xiaojing, Bai, Q. & Lu, Q. 2021b. MiR-30a-5p inhibits osteogenesis and promotes periodontitis by targeting Runx2. *BioMed Central Oral Health*, 21(1): 513.

Liu, Y., Liu, L., Jia, Y., Sun, Y. & Ma, F. 2019. Role of microRNA-15a-5p in the atherosclerotic inflammatory response and arterial injury improvement of diabetic by targeting FASN. *Bioscience Reports*, 39(7): BSR20181852.

Livak, K.J. & Schmittgen, T.D. 2001. Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the 2−ΔΔCT Method. *Methods*, 25(4): 402–408.

Lobo, S.M., Orrico, S.R.P., Queiroz, M.M., Cunrath, G.S., Chibeni, G.S.A., Contrin, L.M., Cury, P.M., de Almeida Burdmann, E., de Oliveira Machado, A.M., Togni, P., De Backer, D., Preiser, J.-C., Szabó, C. & Vincent, J.-L. 2005. Pneumonia-induced sepsis and gut injury: Effects of a poly-(ADP-ribose) polymerase inhibitor. *Journal of Surgical Research*, 129(2): 292–297.

Loirand, G., Guérin, P. & Pacaud, P. 2006. Rho kinases in cardiovascular physiology and pathophysiology. *Circulation Research*, 98(3): 322–334.

Loman, N.J., Misra, R. V, Dallman, T.J., Constantinidou, C., Gharbia, S.E., Wain, J. & Pallen, M.J. 2012. Performance comparison of benchtop high-throughput sequencing platforms. *Nature Biotechnology*, 30(5): 434–439.

Lootens, L., Brusselaers, N., Beele, H. & Monstrey, S. 2013. Keratinocytes in the treatment of severe burn injury: an update. *International Wound Journal*, 10(1): 6–12.

López-Sánchez, G.N., Montalvo-Javé, E., Domínguez-Perez, M., Antuna-Puente, B., Beltrán-Anaya, F.O., Hidalgo-Miranda, A., Chávez-Tapia, N.C., Uribe, M. & Nuño- Lámbarri, N. 2022. Hepatic mir-122-3p, mir-140-5p and mir-148b-5p expressions are correlated with cytokeratin-18 serum levels in MAFLD. *Annals of Hepatology*, 27(6): 100756.

Lou, Y. & Huang, Z. 2020. MicroRNA-15a-5p participates in sepsis by regulating the inflammatory response of macrophages and targeting TNIP2. *Experimental & Therapeutic Medicine*, 19(4): 3060–3068.

Lu, T.X., Munitz, A. & Rothenberg, M.E. 2009. MicroRNA-21 is up-regulated in allergic airway inflammation and regulates IL-12p35 expression. *The Journal of Immunology*, 182(8): 4994–5002.

Lu, X., Yu, Y. & Tan, S. 2019. The role of the miR-21-5p-mediated inflammatory pathway in ulcerative colitis. *Experimental & Therapeutic Medicine*, 19(2): 981–989.

Lu, Y., Thomson, J.M., Wong, H.Y.F., Hammond, S.M. & Hogan, B.L.M. 2007. Transgenic over-expression of the microRNA miR-17-92 cluster promotes proliferation and inhibits differentiation of lung epithelial progenitor cells. *Developmental Biology*, 310(2): 442–453.

Lu, Z., Ghosh, S., Wang, Z. & Hunter, T. 2003. Downregulation of caveolin-1 function by EGF leads to the loss of E-cadherin, increased transcriptional activity of β-catenin, and enhanced tumor cell invasion. *Cancer Cell*, 4(6): 499–515.

Luan, G., Wang, M., Yuan, J., Bu, X., Wang, Y., Ying, S., Wang, C. & Zhang, L. 2022. MicroRNA-21-5p promotes mucosal type 2 inflammation via regulating GLP1R/IL-33 signaling in chronic rhinosinusitis with nasal polyps. *Journal of Allergy & Clinical Immunology*, 150(6): 1460–1475.

Luan, X., Zhou, X., Naqvi, A., Francis, M., Foyle, D., Nares, S. & Diekwisch, T.G.H. 2018. MicroRNAs and immunity in periodontal health and disease. *International Journal of Oral Science*, 10(3): 24.

Lund, T., Goodwin, C.W., McManus, W.F., Shirani, K.Z., Stallings, R.J., Mason Jr, A.D. & Pruitt Jr, B.A. 1985. Upper airway sequelae in burn patients requiring endotracheal intubation or tracheostomy. *Annals of Surgery*, 201(3): 374–382.

Luo, S. & Rubinsztein, D.C. 2013. BCL2L11/BIM. *Autophagy*, 9(1): 104–105.

Luo, X., Budihardjo, I., Zou, H., Slaughter, C. & Wang, X. 1998. Bid, a Bcl2 interacting protein, mediates cytochrome c release from mitochondria in response to activation of cell surface death receptors. *Cell*, 94(4): 481–490.

Lv, L.-L., Cao, Y., Liu, D., Xu, M., Liu, H., Tang, R.-N., Ma, K.-L. & Liu, B.-C. 2013. Isolation and quantification of microRNAs from urinary exosomes/microvesicles for biomarker discovery. *International Journal of Biological Sciences*, 9(10): 1021–1031.

Lv, X., Niu, Q., Zhang, M., Feng, L. & Feng, J. 2021. Critical functions of microRNA-30a-5p-E2F3 in cardiomyocyte apoptosis induced by hypoxia/reoxygenation. *The Kaohsiung Journal of Medical Sciences*, 37(2): 92–100.

Lyu, H.-P., Cheng, M., Liu, J.-C., Ye, M.-Y., Xu, D., He, J.-T., Xie, X.-L. & Wang, Q. 2018. Differentially expressed microRNAs as potential markers for vital reaction of burned skin. *Journal of Forensic Science & Medicine*, 4(3): 135–141.

Ma, B. & Hottiger, M.O. 2016. Crosstalk between Wnt/β-Catenin and NF-κB signaling pathway during inflammation. *Frontiers in Immunology*, 7: 378.

Ma, J., Gan, L., Chen, H., Chen, L., Hu, Y., Luan, C., Chen, K. & Zhang, J. 2024. Upregulated miR-374a-5p drives psoriasis pathogenesis through WIF1 downregulation and Wnt5a/NF-κB activation. *Cellular Signalling*, 119: 111171.

Ma, Z., Lee, R.W., Li, B., Kenney, P., Wang, Y., Erikson, J., Goyal, S. & Lao, K. 2013. Isothermal amplification method for next-generation sequencing. *Proceedings of the National Academy of Sciences*, 110(35): 14320 – 14323.

Mabrouk, A., Maher, A. & Nasser, S. 2003. An epidemiologic study of elderly burn patients in Ain Shams University Burn Unit, Cairo, Egypt. *Burns*, 29(7): 687–690.

MacFarlane, L.-A. & Murphy, P. R. 2010. MicroRNA: Biogenesis, function and role in cancer. *Current Genomics*, 11(7): 537–561.

Machhar, R., Pollock, R., Ye, J., Chandran, V. & Gladman, D.D. 2019. FRI0358 role of miRNA-21-5PAS a potential biomarker for the inflammatin pathway in psoriatic disease and response to methotrexate treatment. *Annals of the Rheumatic Diseases*, 78: 861.

Mack, E. 2007. Co-oximetry. *Pediatrics in Review*, 28(2): 73.

Mackay, A. & Al-Haddad, M. 2009. Acute lung injury and acute respiratory distress syndrome. *Continuing Education in Anaesthesia, Critical Care & Pain*, 9(5): 152–156.

Mackutwa, E., Khainga, S., Ndung'u, J. & Anangwe, C. 2021. Significance of Early Acidosis in Predicting Early Mortality Among Flame Burned Patients in a Kenyan National Hospital. *East African Medical Journal*, 100(3).

Mallick, B., Ghosh, Z. & Chakrabarti, J. 2009. MicroRNome analysis unravels the molecular basis of SARS infection in bronchoalveolar stem cells. *PLoS One*, 4(11): e7837.

Mar-Aguilar, F., Mendoza-Ramírez, J.A., Malagón-Santiago, I., Espino-Silva, P.K., Santuario-Facio, S.K., Ruiz-Flores, P., Rodríguez-Padilla, C. & Reséndez-Pérez, D. 2013. Serum circulating microRNA profiling for identification of potential breast cancer biomarkers. *Disease Markers*, 34(3): 163–169.

Marine, R.L., Magaña, L.C., Castro, C.J., Zhao, K., Montmayeur, A.M., Schmidt, A., Diez-Valcarce, M., Ng, T.F.F., Vinjé, J., Burns, C.C., Nix, W.A., Rota, P.A. & Oberste, M.S. 2020. Comparison of Illumina MiSeq and the Ion Torrent PGM and S5 platforms for whole-genome sequencing of picornaviruses and caliciviruses. *Journal of Virological Methods*, 280: 113865.

Mariner, P.D., Korst, A., Karimpour-Fard, A., Stauffer, B.L., Miyamoto, S.D. & Sucharov, C.C. 2018. Improved detection of circulating miRNAs in serum and plasma following rapid heat/freeze cycling. *MicroRNA*, 7(2): 138–147.

Maritz, D., Wallis, L., Van Der Merwe, E. & Nel, D. 2012. The aetiology of adult burns in the

Western Cape, South Africa. *Burns*, 38(1): 120–127.

Martinez, F.O., Helming, L., Milde, R., Varin, A., Melgert, B.N., Draijer, C., Thomas, B., Fabbri, M., Crawshaw, A., Ho, L.P., Ten Hacken, N.H., Cobos Jiménez, V., Kootstra, N.A., Hamann, J., Greaves, D.R., Locati, M., Mantovani, A. & Gordon, S. 2013. Genetic programs expressed in resting and IL-4 alternatively activated mouse and human macrophages: similarities and differences. *Blood*, 121(9): e57–e69.

Martinez, N.J. & Gregory, R.I. 2013. Argonaute2 expression is post-transcriptionally coupled to microRNA abundance. *RNA*, 19(5): 605–612.

Maruo, N., Morita, I., Shirao, M. & Murota, S. 1992. IL-6 increases endothelial permeability in vitro. *Endocrinology*, 131(2): 710–714.

Masanès, M.-J., Legendre, C., Lioret, N., Saizy, R. & Lebeau, B. 1995. Using bronchoscopy and biopsy to diagnose early inhalation injury: Macroscopic and histologic findings. *Chest*, 107(5): 1365–1369.

Mascia, F., Mariani, V., Girolomoni, G. & Pastore, S. 2003. Blockade of the EGF receptor induces a deranged chemokine expression in keratinocytes leading to enhanced skin inflammation. *The American Journal of Pathology*, 163(1): 303–312.

Mashavave, N.Z., Withers, A., Doedens, L., Jugmohan, B. & Loveland, J. 2020. A retrospective study analysing mortality and outcomes in the paediatric burns intensive care unit at the Chris Hani Baragwanath Academic Hospital, Johannesburg. *South African Journal of Child Health*, 14(4): 212.

Mathelier, A. & Carbone, A. 2010. MIReNA: finding microRNAs with high accuracy and no learning at genome scale and from deep sequencing data. *Bioinformatics*, 26(18): 2226–2234.

Mathivanan, S., Ji, H. & Simpson, R.J. 2010. Exosomes: Extracellular organelles important in intercellular communication. *Journal of Proteomics*, 73(10): 1907–1920.

Mathonsi, K. & Arko-Cobbah, E. 2023. Outcomes of burns patients in a developing country: A single centre's experience. *South African Medical Journal*, 113(10): 49–53.

Matthay, M.A., Ware, L.B. & Zimmerman, G.A. 2012. The acute respiratory distress syndrome. *The Journal of Clinical Investigation*, 122(8): 2731–2740.

Matute-Bello, G., Liles, W.C., Steinberg, K.P., Kiener, P.A., Mongovin, S., Chi, E.Y., Jonas, M. & Martin, T.R. 1999. Soluble Fas ligand induces epithelial cell apoptosis in humans with acute lung injury (ARDS). *Journal of Immunology*, 163(4): 2217–2225.

Matzopoulos, R. 2005. *A Profile of Fatal Injuries in South Africa: 6th Annual Report of the National Injury Mortality Surveillance System, 2004*. http://196.21.144.194/crime/national2004.pdf [4 August 2021].

McCall, J.E. & Cahill, T.J. 2005. Respiratory care of the burn patient. *Journal of Burn Care & Research*, 26(3): 200–206.

McDonald, J.S., Milosevic, D., Reddi, H. V, Grebe, S.K. & Algeciras-Schimnich, A. 2011. Analysis of circulating microRNA: Preanalytical and analytical challenges. *Clinical Chemistry*, 57(6): 833–840.

Mcgwin, G., George, R., Cross, J., Reiff, D., Chaudry, I. & Rue, L. 2002. Gender differences in mortality following burn injury. *Shock*, 18(4): 311–315.

Megahed, M.A., Elhelbawy, R.H., Agha, M.A., Abdelatty, N.B., El-Koa, A.A. & El-Kalashy, M.M. 2023. First-day computed tomography: does it has a role in the assessment of patients with inhalation lung injury? *The Egyptian Journal of Bronchology*, 17(1): 18.

Meghanathan, N. 2016. Assortativity analysis of real-world network graphs based on centrality metrics. *Computer & Information Science*, 9(3): 7.

Meister, G. 2013. Argonaute proteins: functional insights and emerging roles. *Nature Reviews Genetics*, 14(7): 447–459.

Merlani, P., Garnerin, P., Diby, M., Ferring, M. & Ricou, B. 2001. Quality improvement report: Linking guideline to regular feedback to increase appropriate requests for clinical tests: blood gas analysis in intensive care. *British Medical Journal*, 323(7313): 620–624.

Mestdagh, P., Van Vlierberghe, P., De Weer, A., Muth, D., Westermann, F., Speleman, F. & Vandesompele, J. 2009. A novel and universal method for microRNA RT-qPCR data normalization. *Genome Biology*, 10(6): R64.

Meyer, N.J., Huang, Y., Singleton, P.A., Sammani, S., Moitra, J., Evenoski, C.L., Husain, A.N., Mitra, S., Moreno-Vinasco, L., Jacobson, J.R., Lussier, Y.A. & Garcia, J.G.N. 2009. GADD45a is a novel candidate gene in inflammatory lung injury via influences on Akt signaling. *The Federation of American Societies for Experimental Biology Journal*, 23(5): 1325–1337.

Mi, H., Muruganujan, A. & Thomas, P.D. 2012. PANTHER in 2013: modeling the evolution of gene function, and other gene attributes, in the context of phylogenetic trees. *Nucleic Acids Research*, 41(D1): D377–D386.

Mi, H. & Thomas, P. 2009. PANTHER Pathway: An ontology-based pathway database coupled with data analysis tools. *Methods Mol Biol*, 563: 123–140.

Mi, Q.-S., Wang, J., Liu, Q., Wu, X. & Zhou, L. 2021. MicroRNA dynamic expression regulates invariant NKT cells. *Cellular & Molecular Life Science*s, 78(16): 6003–6015.

Miao, X. 2017. Recent advances in understanding the role of miRNAs in exosomes and their therapeutic potential. *Journal of Integrative Agriculture*, 16(4): 753–761.

Milde-Langosch, K., Röder, H., Andritzky, B., Aslan, B., Hemminger, G., Brinkmann, A., Bamberger, C.M., Löning, T. & Bamberger, A.-M. 2004. The role of the AP-1 transcription factors c-Fos, FosB, Fra-1 and Fra-2 in the invasion process of mammary carcinomas. *Breast Cancer Research & Treatment*, 86(2): 139–152.

Millis, S.Z., Ikeda, S., Reddy, S., Gatalica, Z. & Kurzrock, R. 2016. Landscape of phosphatidylinositol-3-kinase pathway alterations across 19 784 diverse solid tumors. The *Journal of the Americal Medicine Association Oncology*, 2(12): 1565.

Miscianinov, V., Martello, A., Rose, L., Parish, E., Cathcart, B., Mitić, T., Gray, G.A., Meloni, M., Al Haj Zen, A. & Caporali, A. 2018. MicroRNA-148b targets the TGF-β pathway to regulate angiogenesis and endothelial-to-mesenchymal transition during skin wound healing. *Molecular Therapy*, 26(8): 1996–2007.

Mishra, R.K., Potteti, H.R., Tamatam, C.R., Elangovan, I. & Reddy, S.P. 2016. c-Jun is required for nuclear factor-κB–dependent, LPS-stimulated Fos-related antigen-1 transcription in alveolar msacrophages. *American Journal of Respiratory Cell & Molecular Biology*, 55(5): 667–674.

Mitchell, P., Parkin, R., Kroh, E., Fritz, B., Wyman, S., Pogosova-Agadjanyan, E., Peterson, A., Noteboom, J., O'Briant, K., Allen, A., Lin, D., Urban, N., Drescher, C., Knudsen, B., Stirewalt, D., Gentleman, R., Vessella, R., Nelson, P., Martin, D. & Tewari, M. 2008. Circulating microRNAs as stable blood-based markers for cancer detection. *Proceedings of the National Academy of Sciences of the United States of America*, 105(30): 10513–10518.

Mlcak, R.P., Suman, O.E. & Herndon, D.N. 2007. Respiratory management of inhalation injury. *Burns*, 33(1): 2–13.

Mohammed, S.N., Amin, M.A., ElHalim, M.M.A. & Nawar, K.I.K. 2023. Evaluation of blood lactate

concentrations as a marker for resuscitation and prognosis in patients with major burns. *QJM: An International Journal of Medicine*, 116(Supplement_1): hcad069.688.

Mokline, A., Abdenneji, A., Rahmani, I., Gharsallah, L., Tlaili, S., Harzallah, I., Gasri, B., Hamouda, R. & Messadi, A.A. 2017. Lactate: prognostic biomarker in severely burned patients. *Annals of Burns & Fire Disasters*, 30(1): 35–38.

Moldovan, L., Batte, K., Wang, Y., Wisler, J. & Piper, M. 2013. Analyzing the circulating microRNAs in exosomes/extracellular vesicles from serum or plasma by qRT-PCR. *Methods in Molecular Biology*, 1024: 129–145.

Molina-Pinelo, S., Suárez, R., Pastor, M.D., Nogal, A., Márquez-Martín, E., Martín-Juan, J., Carnero, A. & Paz-Ares, L. 2012. Association between the miRNA signatures in plasma and bronchoalveolar fluid in respiratory pathologies. *Disease Markers*, 32(4): 221–230.

Monteiro, D., Silva, I., Egipto, P., Magalhães, A., Filipe, R., Silva, A., Rodrigues, A. & Costa, J. 2017. Inhalation injury in a burn unit: a retrospective review of prognostic factors. *Annals of Burns & Fire Disasters*, 30(2): 121–125.

Moody, L., He, H., Pan, Y.-X. & Chen, H. 2017. Methods and novel technology for microRNA quantification in colorectal cancer screening. *Clinical Epigenetics*, 9(1): 119.

Moore, S., Ho, I. & Hume, A. 1991. Severe hypoxia produced by concomitant intoxication with sublethal doses of carbon monoxide and cyanide. *Toxicology & Applied Pharmacology*, 109(3): 412–420.

Morgan, M.J. & Liu, Z. 2011. Crosstalk of reactive oxygen species and NF-κB signaling. *Cell Research*, 21(1): 103–115.

Morimoto, K., Janssen, W.J. & Terada, M. 2012. Defective efferocytosis by alveolar macrophages in IPF patients. *Respiratory Medicine*, 106(12): 1800–1803.

Morobadi, K., Blumenthal, R. & Saayman, G. 2019. Thermal fatalities in Pretoria: A 5-year retrospective review. *Burns*, 45(7): 1707–1714.

Morris, G.P., Rebeiro, L., Herridge, M.S., Szewczuk, M. & Depew, W. 1984. An animal model for chronic granulomatous inflammation of the stomach and colon. *Gastroenterology*, 86: 1188.

Morris, N.L., Hammer, A.M., Cannon, A.R., Gagnon, R.C., Li, X. & Choudhry, M.A. 2017. Dysregulation of microRNA biogenesis in the small intestine after ethanol and burn injury. *Biochimica et Biophysica Acta*, 1863(10, Part B): 2645–2653.

Moschos, S.A., Williams, A.E., Perry, M.M., Birrell, M.A., Belvisi, M.G. & Lindsay, M.A. 2007. Expression profiling in vivo demonstrates rapid changes in lung microRNA levels following lipopolysaccharide-induced inflammation but not in the anti-inflammatory action of glucocorticoids. *BioMed Central Genomics*, 8(1): 240.

Moshrefi, S., Sheckter, C.C., Shepard, K., Pereira, C., Davis, D.J., Karanas, Y. & Rochlin, D.H. 2019. Preventing unnecessary intubations: A 5-Year regional burn center experience using flexible fiberoptic laryngoscopy for airway evaluation in patients with suspected inhalation or airway injury. *Journal of Burn Care & Research*, 40(3): 341–346.

Mosier, M., Pham, T., Park, D., Simmons, J., Klein, M. & Gibran, N. 2012. Predictive value of bronchoscopy in assessing the severity of inhalation injury. *Journal of Burn Care & Research*, 33(1): 65–73.

Motameny, S., Wolters, S., Nürnberg, P. & Schumacher, B. 2010. Next generation sequencing of miRNAs - Strategies, Resources and Methods. *Genes*, 1(1): 70–84.

Moylan, J.A. & Alexander Jr, L.G. 1978. Diagnosis and treatment of inhalation injury. *World Journal*

of Surgery, 2: 185–189.

Mu, S., Kang, B., Zeng, W., Sun, Y. & Yang, F. 2016. MicroRNA-143-3p inhibits hyperplastic scar formation by targeting connective tissue growth factor CTGF/CCN2 via the Akt/mTOR pathway. *Molecular & Cellular Biochemistry*, 416(1–2): 99–108.

Muehlberger, T., Kunar, D., Munster, A. & Couch, M. 1998. Efficacy of Fiberoptic Laryngoscopy in the Diagnosis of Inhalation Injuries. *Archives of Otolaryngology*, 124(9): 1003–1007.

Mueller, O. 2004. RNA Integrity Number (RIN). *Nano*: 1–17.

Mukaka, M.M. 2012. Statistics corner: A guide to appropriate use of correlation coefficient in medical research. *Malawi Medical Journal*, 24(3): 69–71.

Mukherjee, S.P., Behar, M., Birnbaum, H.A., Hoffmann, A., Wright, P.E. & Ghosh, G. 2013. Analysis of the RelA:CBP/p300 Interaction Reveals Its Involvement in NF-κB-Driven Transcription. *PLoS Biology*, 11(9): e1001647.

Mulay, S.R., Thomasova, D., Ryu, M. & Anders, H.-J. 2012. MDM2 (murine double minute-2) links inflammation and tubular cell healing during acute kidney injury in mice. *Kidney International*, 81(12): 1199–1211.

Mulcahy, L.A., Pink, R.C. & Carter, D.R.F. 2014. Routes and mechanisms of extracellular vesicle uptake. *Journal of Extracellular Vesicles*, 3(1): 24641.

Muller, M., Pegg, S. & Rule, M. 2001. Determinants of death following burn injury. *The British Journal of Surgery*, 88(4): 583–587.

Munich, S., Sobo-Vujanovic, A., Buchser, W.J., Beer-Stolz, D. & Vujanovic, N.L. 2012. Dendritic cell exosomes directly kill tumor cells and activate natural killer cells via TNF superfamily ligands. *Oncoimmunology*, 1(7): 1074–1083.

Murakami, K. & Traber, D.L. 2003. Pathophysiological basis of smoke inhalation injury. *News in Physiological Sciences*, 18(3): 125–129.

Muthukumar, V., Arumugam, P.K., Narasimhan, A., Kumar, S., Sharma, U., Sharma, S. & Kain, R. 2020. Blood lactate and lactate clearance: Refined biomarker and prognostic marker in burn resuscitation. *Annals of Burns & Fire Disasters*, 33(4): 293–298.

Mzezewa, S., Jonsson, K., Aberg, M. & Salemark, L. 1999. A prospective study on the epidemiology of burns in patients admitted to the Harare burn units. *Burns*, 25(6): 499–504.

Naidich, D.P., Gruden, J.F., McGuinness, G., McCauley, D.I. & Bhalla, M. 1997. Volumetric (Helical/Spiral) CT (VCT) of the airways. *Journal of Thoracic Imaging*, 12(1): 11–28.

Naqvi, A.R., Khan, S.H., Mittal, D., Alshaya, H., Kazi, M. & Sarwat, M. 2022. Time-dependent oscillation of pre-MiRNAs, mature MiRNAs and MiRNA targets in tomato plants subjected to varying abiotic stress conditions. *Saudi Journal of Biological Sciences*: 103157 (In press, corrected proof).

Ng, E.K.O., Chong, W.W.S., Jin, H., Lam, E.K.Y., Shin, V.Y., Yu, J., Poon, T.C.W., Ng, S.S.M. & Sung, J.J.Y. 2009. Differential expression of microRNAs in plasma of patients with colorectal cancer: a potential marker for colorectal cancer screening. *Gut*, 58(10): 1375–1381.

Nie, Z., Hu, G., Wei, G., Cui, K., Yamane, A., Resch, W., Wang, R., Green, D.R., Tessarollo, L., Casellas, R., Zhao, K. & Levens, D. 2012. c-Myc is a universal amplifier of expressed genes in lymphocytes and embryonic stem cells. *Cell*, 151(1): 68–79.

Niu, H., Pang, Y., Xie, L., Yu, Q., Shen, Y., Li, J. & Xu, X. 2023. Clustering pattern and evolution characteristic of microRNAs in grass carp (Ctenopharyngodon idella). *BioMed Central Genomics*, 24(1): 73.

Noferesti, S.S., Sohel, M.M.H., Hoelker, M., Salilew-Wondim, D., Tholen, E., Looft, C., Rings, F., Neuhoff, C., Schellander, K. & Tesfaye, D. 2015. Controlled ovarian hyperstimulation induced changes in the expression of circulatory miRNA in bovine follicular fluid and blood plasma. *Journal of Ovarian Research*, 8(1): 81.

Nunez Lopez, O., Cambiaso-Daniel, J., Branski, L., Norbury, W. & Herndon, D. 2017. Predicting and managing sepsis in burn patients: current perspectives. *Therapeutics & Clinical Risk Management*, 13: 1107–1117.

Nybo, K. 2011. RNA isolation: Improving quality. *BioTechniques*, 50(5): 291–292.

O'Brien, J., Hayder, H., Zayed, Y. & Peng, C. 2018. Overview of microRNA biogenesis, mechanisms of actions, and circulation. *Frontiers in Endocrinology*, 9: 402.

O'Keefe, G.E., Hunt, J.L. & Purdue, G.F. 2001. An evaluation of risk factors for mortality after burn trauma and the identification of gender-dependent differences in outcomes. *Journal of the American College of Surgeons*, 192(2): 153–160.

Oh, A., Pardo, M., Rodriguez, A., Yu, C., Nguyen, L., Liang, O., Chorzalska, A. & Dubielecka, P.M. 2023. NF-κB signaling in neoplastic transition from epithelial to mesenchymal phenotype. *Cell Communication & Signaling*, 21(1): 291.

Oh, J., Chung, K., Allen, A., Batchinsky, A., Huzar, T., King, B., Wolf, S., Sjulin, T. & Cancio, L. 2012. Admission chest CT complements fiberoptic bronchoscopy in prediction of adverse outcomes in thermally injured patients. *Journal of Burn Care & Research*, 33(4): 532–538.

Okada, C., Yamashita, E., Lee, S.J., Shibata, S., Katahira, J., Nakagawa, A., Yoneda, Y. & Tsukihara, T. 2009. A high-resolution structure of the pre-microRNA nuclear export machinery. *Science*, 326(5957): 1275–1279.

Okada, H., Kohanbash, G. & Lotze, M.T. 2010. MicroRNAs in immune regulation--opportunities for cancer immunotherapy. *The International Journal of Biochemistry & Cell biology*, 42(8): 1256–1261.

Okamura, K., Ishizuka, A., Siomi, H. & Siomi, M.C. 2004. Distinct roles for Argonaute proteins in small RNA-directed RNA cleavage pathways. *Genes & Development*, 18(14): 1655–1666.

Omori, K., Mitsuhashi, M., Ishiyama, K., Nair, I., Rawson, J., Todorov, I., Kandeel, F. & Mullen, Y. 2011. mRNA of the pro-apoptotic gene BBC3 serves as a molecular marker for TNF-α-induced islet damage in humans. *Diabetologia*, 54(8): 2056–2066.

Onah, C.N., Allmendinger, R., Handl, J. & Dunn, K.W. 2021. Surviving Burn Injury: Drivers of Length of Hospital Stay. *International Journal of Environmental Research & Public Health*, 18(2): 761.

Onishi, S., Osuka, A., Kuroki, Y. & Ueyama, M. 2017. Indications of early intubation for patients with inhalation injury. *Acute Medicine & Surgery*, 4(3): 278–285.

Oren, M. 2003. Decision making by p53: life, death and cancer. *Cell Death & Differentiation*, 10(4): 431–442.

Orozco-Peláez, Y.A. 2018. Airway burn or inhalation injury: should all patients be intubated? *Colombian Journal of Anesthesiology*, 46(Suppl 1): 26–31.

Osborn, L., Hession, C., Tizard, R., Vassallo, C., Luhowskyj, S., Chi-Rosso, G. & Lobb, R. 1989. Direct expression cloning of vascular cell adhesion molecule 1, a cytokine-induced endothelial protein that binds to lymphocytes. *Cell*, 59(6): 1203–1211.

Osler, T., Glance, L. & Hosmer, D. 2010. Simplified estimates of the probability of death after burn injuries: Extending and updating the Baux Score. *The Journal of Trauma*, 68(3): 690–697.

Ouyang, T., Liu, Z., Han, Z. & Ge, Q. 2019. MicroRNA detection specificity: Recent advances and future perspective. *Analytical Chemistry*, 91(5): 3179–3186.

Palmieri, T.L. & Gamelli, R.L. 2012. Diagnosis and management of inhalation injury. In M. G. Jeschke, L.-P. Kamolz, F. Sjöberg, & S. E. Wolf, eds. *Handbook of Burns*. Vienna: Springer: 163–172.

Palmieri, T.L. & Klein, M.B. 2007. Burn research state of the science: Introduction. *Journal of Burn Care & Research*, 28(4): 544–545.

Pan, X., Wenzel, A., Jensen, L.J. & Gorodkin, J. 2018. Genome-wide identification of clusters of predicted microRNA binding sites as microRNA sponge candidates. *PLoS One*, 13(8): e0202369.

Panjeshahin, M.-R., Lari, A.R., Talei, A.-R., Shamsnia, J. & Alaghehbandan, R. 2001. Epidemiology and mortality of burns in the South West of Iran. *Burns*, 27(3): 219–226.

Paquette, A., Shynlova, O., Wu, X., Kibschull, M., Wang, K., Price, N. & Lye, S. 2019. MicroRNA-transcriptome networks in whole blood and monocytes of women undergoing preterm labour. *Journal of Cellular & Molecular Medicine*, 23(10): 6835– 6845.

Parbhoo, A., Louw, Q.A. & Grimmer-somers, K. 2010. A profile of hospital-admitted paediatric burns patients in South Africa. *BioMed Central Research Notes*, 3(1): 165.

Park, H.S., Kim, S.R. & Lee, Y.C. 2009. Impact of oxidative stress on lung diseases. *Respirology*, 14(1): 27–38.

Park, J.H., Shin, S.-Y. & Shin, C. 2017. Non-canonical targets destabilize microRNAs in human Argonautes. *Nucleic Acids Research*, 45(4): gkx029.

Park, M., Cancio, L., Batchinsky, A., McCarthy, M., Jordan, B., Brinkley, W., Dubick, M. & Goodwin, C.O.L. 2003. Assessment of severity of ovine smoke inhalation injury by analysis of computed tomographic scans. *The Journal of Trauma*, 55(3): 417–427.

Park, M.S., Cancio, L.C., Jordan, B.S., Brinkley, W.W., Rivera, V.R. & Dubick, M.A. 2004. Assessment of oxidative stress in lungs from sheep after inhalation of wood smoke. *Toxicology*, 195(2–3): 97–112.

Park, S., Kim, C.H., Hyun, I.G. & Jung, K.S. 2006. Diagnosis of acid-base imbalance by Stewart's physicochemical approach and mortality prediction in severe burn patients with inhalation injury. *Korean Journal of Critical Care Medicine*, 21(1): 17–27.

Park, Y.J., Kim, D.H., Kim, S.C., Kim, T.Y., Kang, C., Lee, S.H., Jeong, J.H., Lee, S.B. & Lim, D. 2018. Serum lactate upon emergency department arrival as a predictor of 30-day in-hospital mortality in an unselected population. *PLoS One*, 13(1): e0190519.

Pascut, D., Krmac, H., Gilardi, F., Patti, R., Calligaris, R., Crocè, L.S. & Tiribelli, C. 2019. A comparative characterization of the circulating miRNome in whole blood and serum of HCC patients. *Scientific Reports*, 9(1): 8265.

Patnaik, S.K., Yendamuri, S., Kannisto, E., Kucharczuk, J.C., Singhal, S. & Vachani, A. 2012. MicroRNA expression profiles of whole blood in lung adenocarcinoma. *PLoS One*, 7(9): e46045.

Pattarayan, D., Thimmulappa, R.K., Ravikumar, V. & Rajasekaran, S. 2018. Diagnostic potential of extracellular microRNA in respiratory diseases. *Clinical Reviews in Allergy & Immunology*, 54(3): 480–492.

Paul, P., Chakraborty, A., Sarkar, D., Langthasa, M., Rahman, M., Bari, M., Singha, R.S., Malakar, A.K. & Chakraborty, S. 2018. Interplay between miRNAs and human diseases. *Journal of Cellular Physiology*, 233(3): 2007–2018.

Pearson, G., Robinson, F., Beers Gibson, T., Xu, B., Karandikar, M., Berman, K. & Cobb, M.H. 2001.

Mitogen-activated protein (MAP) kinase pathways: Regulation and physiological functions. *Endocrine Reviews*, 22(2): 153–183.

Pearson, K. 1897. Mathematical contributions to the theory of evolution.—On a form of spurious correlation which may arise when indices are used in the measurement of organs. *Proceedings of the Royal Society of London*, 60(359–367): 489–498.

Peck, M.D. 2011. Epidemiology of burns throughout the world. Part I: Distribution and risk factors. *Burns*, 37(7): 1087–1100.

Peck, M.D., Kruger, G.E., van der Merwe, A.E., Godakumbura, W. & Ahuja, R.B. 2008. Burns and fires from non-electric domestic appliances in low and middle income countries: Part I. The scope of the problem. *Burns*, 34(3): 303–311.

Pegg, S.P. 2005. Burn epidemiology in the Brisbane and Queensland area. *Burns*, 31(1): S27–S31.

Peiró-Chova, L., Peña-Chilet, M., López-Guerrero, J.A., García-Giménez, J.L., Alonso-Yuste, E., Burgues, O. & Lluch, A. 2013. High stability of microRNAs in tissue samples of compromised quality. *Virchows Arch*, 463: 765–774.

Pello, O.M., De Pizzol, M., Mirolo, M., Soucek, L., Zammataro, L., Amabile, A., Doni, A., Nebuloni, M., Swigart, L.B., Evan, G.I., Mantovani, A. & Locati, M. 2012. Role of c-MYC in alternative activation of human macrophages and tumor-associated macrophage biology. *Blood*, 119(2): 411–421.

Perconti, G., Rubino, P., Contino, F., Bivona, S., Bertolazzi, G., Tumminello, M., Feo, S., Giallongo, A. & Coronnello, C. 2019. RIP-Chip analysis supports different roles for AGO2 and GW182 proteins in recruiting and processing microRNA targets. *BioMed Central Bioinformatics*, 20(Suppl 4): 120.

Perez-Sanchez, C., Barbera Betancourt, A., Lyons, P.A., Zhang, Z., Suo, C., Lee, J.C., McKinney, E.F., Modis, L.K., Ellson, C. & Smith, K.G.C. 2022. MiR-374a-5p regulates inflammatory genes and monocyte function in patients with inflammatory bowel disease. *Journal of Experimental Medicine*, 219(5): e20211366.

Perkins, G.D., McAuley, D.F., Giles, S., Routledge, H. & Gao, F. 2003. Do changes in pulse oximeter oxygen saturation predict equivalent changes in arterial oxygen saturation? *Critical Care*, 7(4): R67–R67.

Pernaute, B., Spruce, T., Rodriguez, T.A. & Manzanares, M. 2011. MiRNA-mediated regulation of cell signaling and homeostasis in the early mouse embryo. *Cell Cycle*, 10(4): 584–591.

Petejova, N., Martinek, A., Zadrazil, J., Klementa, V., Pribylova, L., Bris, R., Kanova, M., Sigutova, R., Kacirova, I., Svagera, Z., Bace, E. & Stejskal, D. 2022. Expression and 7-day time course of circulating microRNAs in septic patients treated with nephrotoxic antibiotic agents. *BioMed Central Nephrology*, 23(1): 111.

Peters, L. & Meister, G. 2007. Argonaute proteins: mediators of RNA silencing. *Molecular Cell*, 26(5): 611–623.

Peters, W.J. 1981. Inhalation injury caused by the products of combustion. *Canadian Medical Association Journal*, 125(3): 249–252.

Pham, T.-H., Park, H.-M., Kim, J., Hong, J.T. & Yoon, D.-Y. 2020. STAT3 and p53: Dual Target for Cancer Therapy. *Biomedicines*, 8(12): 637.

Piatkowski, A., Ulrich, D., Grieb, G. & Pallua, N. 2009. A new tool for the early diagnosis of carbon monoxide intoxication. *Inhalation Toxicology*, 21(13): 1144–1147.

Piccinni, P., Dan, M., Barbacini, S., Carraro, R., Lieta, E., Marafon, S., Zamperetti, N., Brendolan, A.,

D'Intini, V., Tetta, C., Bellomo, R. & Ronco, C. 2006. Early isovolaemic haemofiltration in oliguric patients with septic shock. *Intensive Care Medicine*, 32(1): 80–86.

Pilon, C.S., Leathley, M., London, R., McLean, S., Phang, P.T., Priestley, R., Rosenberg, F.M., Singer, J., Anis, A.H. & Dodek, P.M. 1997. Practice guideline for arterial blood gas measurement in the intensive care unit decreases numbers and increases appropriateness of tests. *Critical Care Medicine*, 25(8): 1308–1313.

Pober, J.S. & Cotran, R.S. 1990. The role of endothelial cells in inflammation. *Transplantation*, 50(4): 537–544.

Pottelberge, G.R. Van, Mestdagh, P., Bracke, K.R., Thas, O., Durme, Y.M.T.A. van, Joos, G.F., Vandesompele, J. & Brusselle, G.G. 2011. MicroRNA expression in induced sputum of smokers and patients with chronic obstructive pulmonary disease. *American Journal of Respiratory & Critical Care Medicine*, 183(7): 898–906.

Preston, G.C., Sinclair, L. V, Kaskar, A., Hukelmann, J.L., Navarro, M.N., Ferrero, I., MacDonald, H.R., Cowling, V.H. & Cantrell, D.A. 2015. Single cell tuning of Myc expression by antigen receptor signal strength and interleukin-2 in T lymphocytes. *The European Molecular Biology Organization Journal*, 34(15): 2008–2024.

Pretto, J.J., Roebuck, T., Beckert, L. & Hamilton, G. 2014. Clinical use of pulse oximetry: Official guidelines from the Thoracic Society of Australia and New Zealand. *Respirology*, 19(1): 38–46.

Prien, T. & Traber, D.L. 1988. Toxic smoke compounds and inhalation injury-a review. *Burns*, 14(6): 451–460.

Pritchard, C.C., Kroh, E., Wood, B., Arroyo, J.D., Dougherty, K.J., Miyaji, M.M., Tait, J.F. & Tewari, M. 2012. Blood cell origin of circulating microRNAs: a cautionary note for cancer biomarker studies. *Cancer Prevention Research*, 5(3): 492–497.

Puchta, M., Boczkowska, M. & Groszyk, J. 2020. Low RIN value for RNA-seq library construction from long-term stored seeds: A Case Study of Barley Seeds. *Genes*, 11(10): 1190.

Puneet, P., Hegde, A., Ng, S.W., Lau, H.Y., Lu, J., Moochhala, S.M. & Bhatia, M. 2006. Preprotachykinin-A gene products are key mediators of lung injury in polymicrobial sepsis. *The Journal of Immunology*, 176(6): 3813–3820.

Qin, H., Wilson, C.A., Lee, S.J., Zhao, X. & Benveniste, E.N. 2005. LPS induces CD40 gene expression through the activation of NF-κB and STAT-1α in macrophages and microglia. *Blood*, 106(9): 3114–3122.

Qin, S., Wang, H., Liu, G., Mei, H. & Chen, M. 2019. miR-21-5p ameliorates hyperoxic acute lung injury and decreases apoptosis of AECII cells via PTEN/AKT signaling in rats. *Molecular Medicine Reports*, 20(6): 4953–4962.

Qu, W., Tian, D., Guo, Z., Fang, J., Zhang, Q., Yu, Z., Xie, M., Zhang, H., Lü, J. & Wang, W. 2012. Inhibition of EGFR/MAPK signaling reduces microglial inflammatory response and the associated secondary damage in rats after spinal cord injury. *Journal of Neuroinflammation*, 9(1): 642.

Quail, M.A., Smith, M., Coupland, P., Otto, T.D., Harris, S.R., Connor, T.R., Bertoni, A., Swerdlow, H.P. & Gu, Y. 2012. A tale of three next generation sequencing platforms: comparison of Ion Torrent, Pacific Biosciences and Illumina MiSeq sequencers. *BioMed Central Genomics*, 13(1): 341.

Quan, X., Li, X., Yin, Z., Ren, Y. & Zhou, B. 2019. p53/miR-30a-5p/ SOX4 feedback loop mediates cellular proliferation, apoptosis, and migration of non-small-cell lung cancer. *Journal of Cellular Physiology*, 234(12): 22884–22895.

Quinn, L., Ahmed, T., Falk, H., Miranda Altamirano, A., Muganza, A., Nakarmi, K., Nawar, A., Peck, M., Man Rai, S., Sartori, J., Philipe Molina Vana, L., Wabwire, B., Moiemen, N. & Lilford, R. 2023. Burn Admissions Across Low- and Middle-income Countries: A Repeated Cross-sectional Survey. *Journal of Burn Care & Research*, 44(2): 320–328.

R Core Team. 2011. *R: A language and environment for statistical computing*. http://www.r-project.org/ [11 November 2023].

Radom-Aizik, S., Zaldivar Jr, F., Leu, S.-Y., Adams, G.R., Oliver, S. & Cooper, D.M. 2012. Effects of exercise on microRNA expression in young males peripheral blood mononuclear cells. *Clinical & Translational Science*, 5(1): 32–38.

Rainen, L., Oelmueller, U., Jurgensen, S., Wyrich, R., Ballas, C., Schram, J., Herdman, C., Bankaitis-Davis, D., Nicholls, N., Trollinger, D. & Tryon, V. 2002. Stabilization of mRNA expression in whole blood samples. *Clinical Chemistry*, 48(11): 1883–1890.

Rajewsky, N. 2006. Microrna target predictions in animals. *Nature Genetics*, 38(6S): S8–S13.

Ramabulana, A.-T., Steenkamp, P.A., Madala, N.E. & Dubery, I.A. 2020. Profiling of Altered Metabolomic States in Bidens pilosa Leaves in Response to Treatment by Methyl Jasmonate and Methyl Salicylate. *Plants*, 9(10): 1275.

Ranganathan, K. & Sivasankar, V. 2014. MicroRNAs - Biology and clinical applications. *Journal of Oral & Maxillofacial Pathology*, 18(2): 229–234.

Ranu, H., Wilde, M. & Madden, B. 2011. Pulmonary Function Tests. *Ulster Medical Journal*, 80(2): 84–90.

Rapsang, A.G. & Shyam, D.C. 2014. Scoring systems in the intensive care unit: A compendium. *Indian Journal of Critical Care Medicine*, 18(4): 220–228.

Rathmell, J.C. & Thompson, C.B. 2002. Pathways of apoptosis in lymphocyte development, homeostasis, and disease. *Cell*, 109(2): S97–S107.

Ravage, Z.B., Gomez, H.F., Czermak, B.J., Watkins, S.A. & Till, G.O. 1998. Mediators of microvascular injury in dermal burn wounds. *Inflammation*, 22(6): 619–629.

Ravi, R., Mookerjee, B., van Hensbergen, Y., Bedi, G.C., Giordano, A., El-Deiry, W.S., Fuchs, E.J. & Bedi, A. 1998. p53-mediated repression of nuclear factor-kappaB RelA via the transcriptional integrator p300. *Cancer Research*, 58(20): 4531–4536.

Rawal, G., Yadav, S. & Kumar, R. 2018. Acute Respiratory Distress Syndrome: An Update and Review. *Journal of Translational Internal Medicine*, 6(2): 74–77.

Rech, M.A., Mosier, M.J., McConkey, K., Zelisko, S., Netzer, G., Kovacs, E.J. & Afshar, M. 2019. Outcomes in Burn-Injured Patients Who Develop Sepsis. *Journal of Burn Care & Research*, 40(3): 269–273.

Reedquist, K.A. 2012. Signal transduction pathways in chronic inflammatory autoimmune disease: Small GTPases. *The Open Rheumatology Journal*, 6(1): 259–272.

Rehberg, S., Maybauer, M.O., Enkhbaatar, P., Maybauer, D.M., Yamamoto, Y. & Traber, D.L. 2009. Pathophysiology, management and treatment of smoke inhalation injury. *Expert Review of Respiratory Medicine*, 3(3): 283–297.

Reimertz, C., Kögel, D., Rami, A., Chittenden, T. & Prehn, J.H.M. 2003. Gene expression during ER stress–induced apoptosis in neurons. *The Journal of Cell Biology*, 162(4): 587–597.

Rezaeijo, S.M., Rezaei, M., Poursheikhani, A., Mohammadkhani, S., Goharifar, N., Shayankia, G., Heydarheydari, S., Saberi, A. & Taghizadeh, E. 2023. Integrative bioinformatics analysis of miRNA and mRNA expression profiles identified some potential biomarkers for breast cancer.

Egyptian Journal of Medical Human Genetics, 24(1): 62.

Rice, T.W., Wheeler, A.P., Bernard, G.R., Hayden, D.L., Schoenfeld, D.A. & Ware, L.B. 2007. Comparison of the Spo2/Fio2 Ratio and the Pao2/Fio2 Ratio in Patients With Acute Lung Injury or ARDS. *Chest*, 132(2): 410–417.

Ringo, Y. & Chilonga, K. 2014. Burns at KCMC: Epidemiology, presentation, management and treatment outcome. *Burns*, 40(5): 1024–1029.

Rivals, I., Personnaz, L., Taing, L. & Potier, M.-C. 2007. Enrichment or depletion of a GO category within a class of genes: which test? *Bioinformatics*, 23(4): 401–407.

Roberts, D., Ostryzniuk, P., Loewen, E., Shanks, A., Wasyluk, T., Pronger, L., Hasinoff, I., Roberts, E. & McEwen, T.A.J. 1991. Control of blood gas measurements in intensive-care units. *The Lancet*, 337(8757): 1580–1582.

Robinson, M.D., McCarthy, D.J. & Smyth, G.K. 2010. edgeR : a Bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics*, 26(1): 139–140.

Robinson, M.D. & Oshlack, A. 2010. A scaling normalization method for differential expression analysis of RNA-seq data. *Genome Biology*, 11(3): R25.

Rochlitz, C.F., Heide, I., Thiede, C., Herrmann, R. & de Kant, E. 1995. Evidence for a mutual regulation of p53 and c-myc expression in human colorectal cancer metastases. *Annals of Oncology*, 6(10): 981–986.

Rode, H., Cox, S.G., Numanoglu, A. & Berg, A.M. 2014. Burn care in South Africa: a micro cosmos of Africa. *Pediatric Surgery International*, 30(7): 699–706.

Rode, H., Rogers, A.D., Numanoglu, A., Wallis, L., Allgaier, R., Laflamme, L., Hasselberg, M., Blom, L. & Duvenage, R. 2015. A review of primary and secondary burn services in the Western Cape, South Africa. *South African Medical Journal*, 105(10): 852–857.

Rodriguez, A., Griffiths-Jones, S., Ashurst, J.L. & Bradley, A. 2004. Identification of mammalian microRNA host genes and transcription units. *Genome Research*, 14(10 A): 1902–1910.

Rodriguez, A., Vigorito, E., Clare, S., Warren, M. V., Couttet, P., Soond, D.R., van Dongen, S., Grocock, R.J., Das, P.P., Miska, E.A., Vetrie, D., Okkenhaug, K., Enright, A.J., Dougan, G., Turner, M. & Bradley, A. 2007. Requirement of bic/microRNA-155 for normal immune function. *Science*, 316(5824): 608–611.

Romanowski, K.S., Palmieri, T.L., Sen, S. & Greenhalgh, D.G. 2016. More than one third of intubations in patients transferred to Burn Centers are unnecessary. *Journal of Burn Care & Research*, 37(5): e409–e414.

Rong, Y., Liu, W., Wang, C., Ning, F. & Zhang, G. 2011. Temperature distribution in the upper airway after inhalation injury. *Burns*, 37(7): 1187–1191.

Rooda, I., Hensen, K., Kaselt, B., Kasvandik, S., Pook, M., Kurg, A., Salumets, A. & Velthut-Meikas, A. 2020. Target prediction and validation of microRNAs expressed from FSHR and aromatase genes in human ovarian granulosa cells. *Scientific Reports*, 10(1): 2300.

Rosa, A., Spagnoli, F.M. & Brivanlou, A.H. 2009. The miR-430/427/302 Family controls mesendodermal fate specification via species-specific target selection. *Developmental Cell*, 16(4): 517–527.

Rosen, B.N. & Peterson, L. 1990. Gender differences in children's outdoor play injuries: A review and an integration. *Clinical Psychology Review*, 10(2): 187–205.

Rosenbluth, E.M., Shelton, D.N., Wells, L.M., Sparks, A.E.T. & Van Voorhis, B.J. 2014. Human embryos secrete microRNAs into culture media—a potential biomarker for implantation.

Fertility & Sterility, 101(5): 1493–1500.

Rossato, M., Curtale, G., Tamassia, N., Castellucci, M., Mori, L., Gasperini, S., Mariotti, B., De Luca, M., Mirolo, M., Cassatella, M.A., Locati, M. & Bazzoni, F. 2012. IL-10-induced microRNA-187 negatively regulates TNF-α, IL-6, and IL-12p40 production in TLR4-stimulated monocytes. *Proceedings of the National Academy of Sciences of the United States of America*, 109(45): E3101–E3110.

Rue III, L.W., Cioffi, M.A.J.W.G., Mason, A.D., McManus, C.O.L.W.F. & Pruitt Jr, C.O.L.B.A. 1993. Improved survival of burned patients with inhalation injury. *Archives of Surgery*, 128(7): 772–780.

Rue III, L.W., Cioffi, W.G., Mason Jr., A.D., McManus, W.F. & Pruitt Jr., B.A. 1995. The risk of Pneumonia in thermally injured patients requiring ventilatory support. *The Journal of Burn Care & Rehabilitation*, 16(suppl_3_pt_1): 262–268.

Ryan, C.M., Schoenfeld, D.A., Thorpe, W.P., Sheridan, R.L., Cassem, E.H. & Tompkins, R.G. 1998. Objective estimates of the probability of death from burn injuries. *New England Journal of Medicine*, 338(6): 362–366.

Ryoo, S.M. & Kim, W.Y. 2018. Clinical applications of lactate testing in patients with sepsis and septic shock. *Journal of Emergency & Critical Care Medicine*, 2: 14.

Ryu, H.S., Park, S.-Y., Ma, D., Zhang, J. & Lee, W. 2011. The induction of microRNA targeting IRS-1 is involved in the development of insulin resistance under conditions of mitochondrial Dysfunction in Hepatocytes. *PLoS One*, 6(3): e17343.

Sadeghi, M.S., Rokni, M., Mahmoudi, M. & Farhadi, E. 2023. Ras family signaling pathway in immunopathogenesis of inflammatory rheumatic diseases. *Frontiers in Immunology*, 14: 1151246.

Safari-Alighiarloo, N., Taghizadeh, M., Rezaei-Tavirani, M., Goliaei, B. & Peyvandi, A.A. 2014. Protein-protein interaction networks (PPI) and complex diseases. *Gastroenterology & Hepatology from Bed to Bench*, 7(1): 17–31.

Sahin, E., Haubenwallner, S., Kuttke, M., Kollmann, I., Halfmann, A., Dohnal, A.B., Chen, L., Cheng, P., Hoesel, B., Einwallner, E., Brunner, J., Kral, J.B., Schrottmaier, W.C., Thell, K., Saferding, V., Blüml, S. & Schabbauer, G. 2014. Macrophage PTEN regulates expression and secretion of arginase I modulating innate and adaptive immune responses. *The Journal of Immunology*, 193(4): 1717–1727.

Saia, R.S., Ribeiro, A.B. & Giusti, H. 2020. Cholecystokinin modulates the mucosal inflammatory response and prevents the lipopolysaccharide-induced intestinal epithelial barrier dysfunction. *Shock*, 53(2): 242–251.

Sakurai, H., Schmalstieg, F.C., Traber, L.D., Hawkins, H.K. & Traber, D.L. 1999. Role of L-selectin in physiological manifestations after burn and smoke inhalation injury in sheep. *Journal of Applied Physiology*, 86(4): 1151–1159.

Saleem, A. 2020. A brief review of the next generation sequencing. *Acta Scientific Veterinary Sciences*, 2(10): 2–5.

Salehi, S.H., As'adi, K., Abbaszadeh-Kasbi, A., Isfeedvajani, M.S. & Khodaei, N. 2017. Comparison of six outcome prediction models in an adult burn population in a developing country. *Annals of Burns and Fire Disasters*, 30(1): 13–17.

Salipante, S.J., Kawashima, T., Rosenthal, C., Hoogestraat, D.R., Cummings, L.A., Sengupta, D.J., Harkins, T.T., Cookson, B.T. & Hoffman, N.G. 2014. Performance comparison of Illumina and ion torrent next-generation sequencing platforms for 16S rRNA-based bacterial community profiling. *Applied & Environmental Microbiology*, 80(24): 7583–7591.

Salvador, J.M., Brown-Clay, J.D. & Fornace, A.J. 2013. Gadd45 in stress signaling, cell cycle control, and apoptosis. *Advances in Experimental Medicine Biology*, 793: 1–19.

Salvi, V., Gianello, V., Tiberio, L., Sozzani, S. & Bosisio, D. 2019. Cytokine Targeting by miRNAs in Autoimmune Diseases. *Frontiers in Immunology*, 10: 15.

Samuel, J.C., Campbell, E.L.P., Mjuweni, S., Muyco, A.P., Cairns, B.A. & Charles, A.G. 2011. The epidemiology, management, outcomes and areas for improvement of burn care in Central Malawi: An observational study. *Journal of International Medical Research*, 39(3): 873–879.

Santaniello, J.M., Luchette, F.A., Esposito, T.J., Gunawan, H., Reed, R.L., Davis, K.A. & Gamelli, R.L. 2004. Ten year experience of burn, trauma, and combined burn/trauma injuries comparing outcomes. *The Journal of Trauma*, 57(4): 696–701.

Sasaki, K., Kohanbash, G., Hoji, A., Ueda, R., McDonald, H.A., Reinhart, T.A., Martinson, J., Lotze, M.T., Marincola, F.M., Wang, E., Fujita, M. & Okada, H. 2010. MiR-17-92 expression in differentiated T cells-implications for cancer immunotherapy. *Journal of Translational Medicine*, 8(1): 17.

Sato-Kuwabara, Y., Melo, S., Soares, F. & Calin, G. 2015. The fusion of two worlds: Non-coding RNAs and extracellular vesicles - Diagnostic and therapeutic implications (Review). *International Journal of Oncology*, 46(1): 17–27.

Saunderson, S., Dunn, A., Crocker, P. & McLellan, A. 2014. CD169 mediates the capture of exosomes in spleen and lymph node. *Blood*, 123(2): 208–216.

Schaefer, T.J. & Nunez Lopez, O. 2020. Burn resuscitation and management. In *StatPearls*. Treasure Island (FL): StatPearls Publishing.

Schall, G.L., McDonald, H.D., Carr, L.B. & Capozzi, A. 1978. Xenon ventilation-perfusion lung scans: The early diagnosis of inhalation injury. *Journal of American Medical Association*, 240(22): 2441–2445.

Schmitz, M.L. & Kracht, M. 2016. Cyclin-dependent kinases as coregulators of inflammatory gene expression. *Trends in Pharmacological Sciences*, 37(2): 101–113.

Schöler, N., Langer, C. & Kuchenbauer, F. 2011. Circulating microRNAs as biomarkers - True Blood? *Genome Medicine*, 3(11): 72.

Scholz, C.C., Cavadas, M.A.S., Tambuwala, M.M., Hams, E., Rodríguez, J., Kriegsheim, A. von, Cotter, P., Bruning, U., Fallon, P.G., Cheong, A., Cummins, E.P. & Taylor, C.T. 2013. Regulation of IL-1β–induced NF-κB by hydroxylases links key hypoxic and inflammatory signaling pathways. *Proceedings of the National Academy of Sciences*, 110(46): 18490–18495.

Schreier, B., Gekle, M. & Grossmann, C. 2014. Role of epidermal growth factor receptor in vascular structure and function. *Current Opinion in Nephrology & Hypertension*, 23(2): 113–121.

Schroeder, A., Mueller, O., Stocker, S., Salowsky, R., Leiber, M., Gassmann, M., Lightfoot, S., Menzel, W., Granzow, M. & Ragg, T. 2006. The RIN: An RNA integrity number for assigning integrity values to RNA measurements. *BioMed Central Molecular Biology*, 7: 1–14.

Scrutton, M.C. 1976. Clinical and biochemical aspects of lactic acidosis. *Federation of European Biochemical Societies Letters*, 70(1–2): 294–295.

Sessa, R. & Hata, A. 2013. Role of microRNAs in lung development and pulmonary diseases. *Pulmonary Circulation*, 3(2): 315–328.

Seyed-Forootan, K., Karimi, H., Motevalian, S.A., Momeni, M., Safari, R. & Ghadarjani, M. 2016. LA50 in burn injuries. *Annals of Burns & Fire Disasters*, 29(1): 14–17.

Shankar, G., Naik, V. & Powar, R. 2010. Epidemiolgical study of burn injuries admitted in two

hospitals of north Karnataka. *Indian Journal of Community Medicine*, 35(4): 509.

Sharma, B.R., Harish, D., Singh, V.P. & Bangar, S. 2006. Septicemia as a cause of death in burns: An autopsy study. *Burns*, 32(5): 545–549.

Sharma, N.P., Duke, J.M., Lama, B.B., Thapa, B., Dahal, P., Bariya, N.D., Marston, W. & Wallace, H.J. 2015. Descriptive epidemiology of unintentional burn injuries admitted to a tertiary-level government hospital in Nepal: Gender-specific patterns. *Asia Pacific Journal of Public Health*, 27(5): 551–560.

Shendure, J. & Ji, H. 2008. Next-generation DNA sequencing. *Nature Biotechnology*, 26(10): 1135–1145.

Sheng, Q., Vickers, K., Zhao, S., Wang, J., Samuels, D.C., Koues, O., Shyr, Y. & Guo, Y. 2016. Multi-perspective quality control of Illumina RNA sequencing data analysis. *Briefings in Functional Genomics*, 16(4): 194–204.

Sheppard, N.N., Hemington-Gorse, S., Ghanem, A., Philp, B., Dziewulski, P. & Shelley, O. 2010. The Belgian severity prediction model compared to other scoring systems in a burn intensive care population. *Burns*, 36(8): 1320–1.

Sheppard, N.N., Hemington-Gorse, S., Shelley, O., Philp, B. & Dziewulski, P. 2011. Prognostic scoring systems in burns: A review. *Burns*, 37(8): 1288–1295.

Sheridan, R.L. 2000. Evaluating and managing burn wounds. *Dermatology Nursing*, 12(1): 17–8, 21–8; quiz 30–1.

Sheridan, R.L. 2016. Fire-related inhalation injury. *The New England Journal of Medicine*, 375(5): 464–469.

Shi, C., Liang, Y., Yang, J., Xia, Y., Chen, H., Han, H., Yang, Y., Wu, W., Gao, R. & Qin, H. 2013. MicroRNA-21 knockout improve the survival rate in DSS induced fatal colitis through protecting against inflammation and tissue injury. *PLoS One*, 8(6): e66814.

Shi, R. & Chiang, V.L. 2005. Facile means for quantifying microRNA expression by real-time PCR. *BioTechniques*, 39(4): 519–525.

Shannon, P., Markiel, A., Ozier, O., Baliga, N.S., Wang, J.T., Ramage, D., Amin, N., Schwikowski, B. & Ideker, T. 2003. Cytoscape: A Software Environment for Integrated Models of Biomolecular Interaction Networks. *Genome Research*, 13(11): 2498–2504.

Shiau, Y.-C., Liu, F.-Y., Tsai, J.J.P., Wang, J.-J., Ho, S.-T. & Kao, A. 2003. Usefulness of technetium-99m hexamethylpropylene amine oxime lung scan to detect inhalation lung injury of patients with pulmonary symptoms/signs but negative chest radiograph and pulmonary function test findings after a fire accident—a preliminary report. *Annals of Nuclear Medicine*, 17(6): 435–438.

Shirani, K.Z., Pruitt, B.A. & Mason, A.D. 1987. The influence of inhalation injury and Pneumonia on burn mortality. *Annals of Surgery*, 205(1): 82–87.

Shubert, J. & Sharma, S. 2018. *Inhalation injury*. StatPearls Treasure Island (FL): StatPearls Publishing LLC.

Silva, L., Garcia, L., Oliveira, B., Tanita, M., Festti, J., Cardoso, L., Lavado, L. & Grion, C. 2016. Acute respiratory distress syndrome in burn patients: incidence and risk factor analysis. *Annals of Burns & Fire Disasters*, 29(3): 178–182.

Simons, M. & Raposo, G. 2009. Exosomes – vesicular carriers for intercellular communication. *Current Opinion in Cell Biology*, 21(4): 575–581.

Simpson, R.J., Kalra, H. & Mathivanan, S. 2012. ExoCarta as a resource for exosomal research. *Journal of Extracellular Vesicles*, 1(1): 10.3402/jev.v1i0.18374

Sinex, J.E. 1999. Pulse oximetry: Principles and limitations. *The American Journal of Emergency Medicine*, 17(1): 59–66.

Singh, D., Jash, P.K. & Tyagi, S. 1997. Recent trends in burn mortality in northwest India and its preventive aspects. *Journal of Indian Academy of Forensic Medicine*, 19: 79–88.

Singh, R., Letai, A. & Sarosiek, K. 2019. Regulation of apoptosis in health and disease: the balancing act of BCL-2 family proteins. *Nature Reviews Molecular Cell Biology*, 20(3): 175–193.

Sio, S.W.S., Puthia, M.K., Lu, J., Moochhala, S. & Bhatia, M. 2008. The neuropeptide substance P is a critical mediator of burn-induced acute lung injury. *The Journal of Immunology*, 180(12): 8333–8341.

Sivamuthu, T.C. 2019. Epidemiological study of 100 cases of burn injuries. *International Surgery Journal*, 6(2): 428–431.

Sivasankar, M. & Leydon, C. 2010. The role of hydration in vocal fold physiology. *Current Opinion in Otolaryngology & Head and Beck Surgery*, 18(3): 171–175.

Smith, D.L., Cairns, B.A., Ramadan, F., Dalston, J.S., Fakhry, S.M., Rutledge, R., Meyer, A.A. & Peterson, H.D. 1994. Effect of inhalation injury, burn size, and age on mortality: a study of 1447 consecutive burn patients. *The Journal of Trauma*, 37(4): 655–659.

Smith, M.T.D., Allorto, N.L. & Clarke, D.L. 2016. Modified first world mortality scores can be used in a regional South African burn service with resource limitations. *Burns*, 42(6): 1340–1344.

Smolle, C., Cambiaso-Daniel, J., Forbes, A.A., Wurzer, P., Hundeshagen, G., Branski, L.K., Huss, F. & Kamolz, L.-P. 2017. Recent trends in burn epidemiology worldwide: A systematic review. *Burns*, 43(2): 249–257.

Snel, B. 2000. STRING: a web-server to retrieve and display the repeatedly occurring neighbourhood of a gene. *Nucleic Acids Research*, 28(18): 3442–3444.

Sohel, M.H. 2016. Extracellular/circulating microRNAs: Release mechanisms, functions and challenges. *Achievements in the Life Sciences*, 10(2): 175–186.

Song, C., Xu, Z., Jin, Y., Zhu, M., Wang, K. & Wang, N. 2015. The network of microRNAs, transcription factors, target genes and host genes in human renal cell carcinoma. *Oncology Letters*, 9(1): 498–506.

Song, F., Li, J.-Z., Wu, Y., Wu, W.-Y., Wang, Y. & Li, G. 2021. Ubiquitinated ligation protein NEDD4L participates in MiR-30a-5p attenuated atherosclerosis by regulating macrophage polarization and lipid metabolism. *Molecular Therapy*, 26: 1303–1317.

Song, Y., Mao, B. & Qian, G. 1999. The role of apoptosis and Fas/FasL in lung tissue in patients with acute respiratory distress syndrome. *Chinese Journal of Tuberculosis & Respiratory Diseases*, 22(10): 610–612.

Song, Y., Nie, L., Wang, M., Liao, W., Huan, C., Jia, Z., Wei, D., Liu, P., Fan, K., Mao, Z., Wang, C. & Huo, W. 2022. Differential expression of lncRNA-miRNA-mRNA and their related functional networks in new-onset type 2 Diabetes Mellitus among Chinese rural adults. *Genes*, 13(11): 2073.

Soucy, G., Boivin, G., Labrie, F. & Rivest, S. 2005. Estradiol is required for a proper immune response to bacterial and viral pathogens in the female brain. *The Journal of Immunology*, 174(10): 6391–6398.

Sousse, L.E., Herndon, D.N., Andersen, C.R., Ali, A., Benjamin, N.C., Granchi, T., Suman, O.E. & Mlcak, R.P. 2015. High tidal volume decreases adult respiratory distress syndrome, Aaelectasis, and ventilator days compared with low tidal volume in pediatric burned patients with inhalation

injury. *Journal of the American College of Surgeons*, 220(4): 570–578.

South African Government. 2013. *Government Gazette. No. 4 of 2013: Protection of Personal Information Act, 2013.* https://www.gov.za/sites/default/files/gcis_document/201409/3706726-11act4of2013popi.pdf [9 August 2021].

Souza, R., Jardim, C., Salge, J.M. & Carvalho, C.R.R. 2004. Smoke inhalation injury. *The Brazilian Journal of Pulmonary & International Databases*, 30(5): 557–565.

Speidel, D. 2010. Transcription-independent p53 apoptosis: an alternative route to death. *Trends in Cell Biology*, 20(1): 14–24.

Stark, V.A., Facey, C.O.B., Viswanathan, V. & Boman, B.M. 2021. The Role of miRNAs, miRNA clusters, and isomiRs in development of cancer stem cell populations in colorectal cancer. *International Journal of Molecular Sciences*, 22(3).

Statistics South Africa. 2020. *Quarterly Labour Force Survey - Statistical release P0211*. https://www.statssa.gov.za/publications/P0211/P02111stQuarter2020.pdf [23 June 2021].

Stein, B., Baldwin, A.S., Ballard, D.W., Greene, W.C., Angel, P. & Herrlich, P. 1993. Cross-coupling of the NF-kappa B p65 and Fos/Jun transcription factors produces potentiated biological function. *The European Molecular Biology Organization Journal*, 12(10): 3879–3891.

Steudal, W., Kirmse, M.A.X., Weimann, J., Ullrich, R., Hromi, J. & Zapol, W.M. 2000. Exhaled nitric oxide production by nitric oxide synthase–deficient Mice. *American Journal of Respiratory & Critical Care Medicine*, 162(4 Pt 1): 1262–1267.

Stokowy, T., Eszlinger, M., Świerniak, M., Fujarewicz, K., Jarząb, B., Paschke, R. & Krohn, K. 2014. Analysis options for high-throughput sequencing in miRNA expression profiling. *BioMed Central Research Notes*, 7(1): 144.

Storey, J.D. & Tibshirani, R. 2003. Statistical significance for genomewide studies. *Proceedings of the National Academy of Sciences*, 100(16): 9440–9445.

Suistomaa, M., Ruokonen, E., Kari, A. & Takala, J. 2000. Time-pattern of lactate and lactate to pyruvate ratio in the first 24 hours of intensive care emergency admissions. *Shock*, 14(1): 8–12.

Su, W., Aloi, M.S. & Garden, G.A. 2016. MicroRNAs mediating CNS inflammation: Small regulators with powerful potential. *Brain, Behavior, and Immunity*, 52: 1–8.

Sun, L., Louie, M.C., Vannella, K.M., Wilke, C.A., LeVine, A.M., Moore, B.B. & Shanley, T.P. 2011. New concepts of IL-10-induced lung fibrosis: fibrocyte recruitment and M2 activation in a CCL2/CCR2 axis. *American Journal of Physiology*, 300(3): L341–L353.

Sun, T., Zhao, K., Liu, M., Cai, Z., Zeng, L., Zhang, J., Li, Z. & Liu, R. 2022. miR-30a-5p induces Aβ production via inhibiting the nonamyloidogenic pathway in Alzheimer's disease. *Pharmacological Research*, 178: 106153.

Sundquist, K., Memon, A.A., Palmér, K., Sundquist, J. & Wang, X. 2021. Inflammatory proteins and miRNA-144-5p in patients with depression, anxiety, or stress- and adjustment disorders after psychological treatment. *Cytokine*, 146: 155646.

Sustainable Energy Africa. 2014. *Tackling Urban Energy Poverty in South Africa*. https://www.sustainable.org.za/uploads/files/file72.pdf [18 May 2021].

Suzuki, M., Aikawa, N., Kobayashi, K. & Higuchi, R. 2005. Prognostic implications of inhalation injury in burn patients in Tokyo. *Burns*, 31(3): 331–336.

Symington, I. 1978. Cyanide exposure in fires. *Lancet*, 2(8080): 91–92.

Szabó, C., Salzman, A.L. & Ischiropoulos, H. 1995. Endotoxin triggers the expression of an inducible

isoform of nitric oxide synthase and the formation of peroxynitrite in the rat aorta in vivo. *Federation of European Biochemical Societies Letters*, 363(3): 235–238.

Szklarczyk, D., Franceschini, A., Wyder, S., Forslund, K., Heller, D., Huerta-Cepas, J., Simonovic, M., Roth, A., Santos, A., Tsafou, K.P., Kuhn, M., Bork, P., Jensen, L.J. & von Mering, C. 2015. STRING v10: protein–protein interaction networks, integrated over the tree of life. *Nucleic Acids Research*, 43(D1): D447–D452.

Szklarczyk, D., Gable, A.L., Lyon, D., Junge, A., Wyder, S., Huerta-Cepas, J., Simonovic, M., Doncheva, N.T., Morris, J.H., Bork, P., Jensen, L.J. & Mering, C. von. 2019. STRING v11: protein–protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. *Nucleic Acids Research*, 47(D1): D607–D613.

Tabet, F., Vickers, K.C., Cuesta Torres, L.F., Wiese, C.B., Shoucri, B.M., Lambert, G., Catherinet, C., Prado-Lourenco, L., Levin, M.G., Thacker, S., Sethupathy, P., Barter, P.J., Remaley, A.T. & Rye, K.-A. 2014. HDL-transferred microRNA-223 regulates ICAM-1 expression in endothelial cells. *Nature Communications*, 5(1): 3292.

Tahamtan, A., Teymoori-Rad, M., Nakstad, B. & Salimi, V. 2018. Anti-inflammatory microRNAs and their potential for inflammatory diseases treatment. *Frontiers in Immunology*, 9: 1377.

Tai, C.-F. & Baraniuk, J.N. 2002. Upper airway neurogenic mechanisms. *Current Opinion in Allergy and Clinical Immunology*, 2(1): 11–19.

Talmi, Y.P., Wolf, M., Nusem-Horowitz, S., Bar-Ziv, J. & Kronenberg, J. 1996. Postintubation arytenoid subluxation. *Annals of Otology, Rhinology & Laryngology*, 105(5): 384–390.

Tam, S., de Borja, R., Tsao, M.-S. & McPherson, J.D. 2014. Robust global microRNA expression profiling using next-generation sequencing technologies. *Laboratory Investigation*, 94(3): 350–358.

Tan, H., Yao, H., Lie, Z., Chen, G., Lin, S. & Zhang, Y. 2019. MicroRNA-30a-5p promotes proliferation and inhibits apoptosis of human pulmonary artery endothelial cells under hypoxia by targeting YKL-40. *Molecular Medicine Reports*, 20(1): 236–244.

Tanaka, Y., Shimizu, M. & Hirabayashi, H. 2007. Acute physiology, age, and chronic health evaluation (APACHE) III score is an alternative efficient predictor of mortality in burn patients. *Burns*, 33(3): 316–320.

Tang, D., Chen, M., Huang, X., Zhang, Guicheng, Zeng, L., Zhang, Guangsen, Wu, S. & Wang, Y. 2023. SRplot: A free online platform for data visualization and graphing. *PLoS One*, 18(11): e0294236.

Tang, R., Li, L., Zhu, D., Hou, D., Cao, T., Gu, H., Zhang, J., Chen, J., Zhang, C.-Y. & Zen, K. 2012. Mouse miRNA-709 directly regulates miRNA-15a/16-1 biogenesis at the posttranscriptional level in the nucleus: Evidence for a microRNA hierarchy system. Cell Research, 22(3): 504–515.

Tang, R., She, Q., Lu, Y., Yin, R., Zhu, P., Zhu, L., Zhou, M. & Zheng, C. 2019. Quality control of RNA extracted from PAXgene Blood RNA tubes after different storage periods. *Biopreservation & Biobanking*, 17(5): 477–482.

Tanita, M.T., Capeletti, M.M., Moreira, T.A., Petinelli, R.P., Cardoso, L.T.Q. & Grion, C.M.C. 2020. Risk factors for acute respiratory distress syndrome in severe burns: prospective cohort study. *International Journal of Burns & Trauma*, 10(1): 1–14.

Tanizaki, S. 2015. Assessing inhalation injury in the emergency room. *Open Access Emergency Medicine*, 7: 31–37.

Tao, Y., Chen, Y.-C., Lan, T., Qian, H., Wang, Y. & Jiang, L. 2012. LPS-induced nuclear translocation of RhoA is dependent on NF-κB in the human lung cancer cell line A549.

Oncology Letters, 3(6): 1283–1287.

Taylor, S.L., Sen, S., Greenhalgh, D.G., Lawless, M., Curri, T. & Palmieri, T.L. 2015. A competing risk analysis for hospital length of stay in patients with burns. *Journal of American Medical Association Surgery*, 150(5): 450–456.

Teixidor, H.S., Rubin, E., Novick, G.S. & Alonso, D.R. 1983. Smoke inhalation: radiologic manifestations. *Radiology*, 149(2): 383–387.

Theodorou, P., Spanholtz, T.A., Amini, P., Maurer, C.A., Phan, T.Q. V, Perbix, W., Lefering, R. & Spilker, G. 2009. Cologne burn centre experience with assault burn injuries. *Burns*, 35(8): 1152–1157.

Thom, S., Bhopale, V., Milovanova, T., Hardy, K., Logue, C., Lambert, D., Troxel, A., Ballard, K. & Eisinger, D. 2010. Plasma biomarkers in carbon monoxide poisoning. *Clinical Toxicology*, 48(1): 47–56.

Thomasova, D., Mulay, S.R., Bruns, H. & Anders, H.-J. 2012. p53-independent roles of MDM2 in NF-κB signaling: Implications for cancer therapy, wound healing, and autoimmune diseases. *Neoplasia*, 14(12): 1097–1101.

Thompson, P.B., Herndon, D.N., Traber, D.L. & Abston, S. 1986. Effect on mortality of inhalation injury. *Journal of Trauma and Acute Care Surgery*, 26(2): 163–165.

Tian, T., Zhu, Y.-L., Hu, F.-H., Wang, Y.-Y., Huang, N.-P. & Xiao, Z.-D. 2013. Dynamics of exosome internalization and trafficking. *Journal of Cellular Physiology*, 228(7): 1487–1495.

Tiberio, P., Callari, M., Angeloni, V., Daidone, M.G. & Appierto, V. 2015. Challenges in using circulating miRNAs as cancer biomarkers. *BioMed Research International*,

Tobiasen, J, Hiebert, J.H. & Edlich, R.F. 1982a. Prediction of burn mortality. *Surgery, Gynecology & Obstetrics*, 154(5): 711–714.

Tobiasen, J, Hiebert, J.M. & Edlich, R.F. 1982b. The abbreviated burn severity index. *Annals of Emergency Medicine*, 11(5): 260–262.

Tomankova, T., Petrek, M. & Kriegova, E. 2010. Involvement of microRNAs in physiological and pathological processes in the lung. *Respiratory Research*, 11(1): 159.

Tong, L. & Tergaonkar, V. 2014. Rho protein GTPases and their interactions with NFκB: crossroads of inflammation and matrix biology. *Bioscience Reports*, 34(3): e00115.

Tong, T., Ji, J., Jin, S., Li, X., Fan, W., Song, Y., Wang, M., Liu, Z., Wu, M. & Zhan, Q. 2005. Gadd45a expression induces Bim dissociation from the cytoskeleton and translocation to mitochondria. *Molecular & Cellular Biology*, 25(11): 4488–4500.

Torii, H., Hosoi, J., Beissert, S., Xu, S., Fox, F.E., Asahina, A., Takashima, A., Rook, A.H. & Granstein, R.D. 1997. Regulation of cytokine expression in macrophages and the Langerhans cell-like line XS52 by calcitonin gene-related peptide. *Journal of Leukocyte Biology*, 61(2): 216–223.

Toussaint, J. & Singer, A.J. 2014. The evaluation and management of thermal injuries: 2014 update. *Clinical & Experimental Emergency Medicine*, 1(1): 8–18.

Traber, D.L., Herndon, D.N., Enkhbaatar, P., Maybauer, M.O. & Maybauer, D.M. 2007. The pathophysiology of inhalation injury. In Herndon D.N. (ed.) *Total Burn Care*. Pennsylvania, PA, USA: Saunders Elsevier: 248–261.

Traber, D.L., Herndon, D.N., Enkhbaatar, P., Maybauer, M.O. & Maybauer, D.M. 2012. The pathophysiology of inhalation injury. In *Total Burn Care*. 4th edition. USA: Elsevier Inc: 219-228.e4.

Tredget, E.E., Shankowsky, H.A., Taerum, T. V., Moysa, G.L. & Alton, J.D.M. 1990. The role of inhalation injury in burn trauma A Canadian experience. *Annals of Surgery*, 212(6): 720–727.

Tremblay, C., Albrecht, K.A., Sonke, C.C. & Azad, S. 2024. Approach to burn treatment in the rural emergency department. *Canadian Family Physician*, 70(2): 95–99.

Trillo-Alvarez, C., Cartin-Ceba, R., Kor, D.J., Kojicic, M., Kashyap, R., Thakur, S., Thakur, L., Herasevich, V., Malinchoc, M. & Gajic, O. 2011. Acute lung injury prediction score: derivation and validation in a population-based sample. *European Respiratory Journal*, 37(3): 604 LP – 609.

Tripathi, F.M., Pandey, K., Paul, P.S., Sinha, J.K., Bhattacharya, V. & Bariar, L.M. 1983. Blood gas studies in thermal burns. *Burns*, 10(1): 13–16.

Trotman, L.C. & Pandolfi, P.P. 2003. PTEN and p53: Who will get the upper hand? *Cancer Cell*, 3(2): 97–99.

Truesdell, S.S., Mortensen, R.D., Seo, M., Schroeder, J.C., Lee, J.H., LeTonqueze, O. & Vasudevan, S. 2012. MicroRNA-mediated mRNA translation activation in quiescent cells and oocytes involves recruitment of a nuclear microRNP. *Scientific Reports*, 2: 842.

Trunkey, D.D. 1978. Inhalation Injury. *Surgical Clinics of North America*, 58(6): 1133–1140.

Tsurumi, A., Que, Y.-A., Yan, S., Tompkins, R.G., Rahme, L.G. & Ryan, C.M. 2015. Do standard burn mortality formulae work on a population of severely burned children and adults? *Burns*, 41(5): 935–945.

Tüfekci, K.U., Öner, M.G., Meuwissen, R.L.J. & Genç, Ş. 2014. The role of microRNAs in human diseases. In Yousef . M. & Allmer, J. (eds.) *miRNomics: MicroRNA Biology and Computational Analysis. Methods in Molecular Biology (Methods and Protocols).* Totowa, NJ: Humana Press: 33–50.

Türegün, M., Sengezer, M., Selmanpakoglu, N., Çeliköz, B. & Nişanci, M. 1997. The last 10 years in a burn centre in Ankara, Turkey: an analysis of 5264 cases. *Burns*, 23(7): 584–590.

Tusavitz, S., Keoonela, S., Kalkstein, M., McCormick, S., Gasser, B., Arrigale, M., Rafferty, P. & Carpenter, A.C. 2020. Macrophage-derived Wnt signaling increases endothelial permeability during skeletal muscle injury. *Inflammation Research*, 69(12): 1235–1244.

Tyson, A.F., Boschini, L.P., Kiser, M.M., Samuel, J.C., Mjuweni, S.N., Cairns, B.A. & Charles, A.G. 2013. Survival after burn in a sub-Saharan burn unit: Challenges and opportunities. *Burns*, 39(8): 1619–1625.

Uda, S., Spolitu, S., Angius, F., Collu, M., Accossu, S., Banni, S., Murru, E., Sanna, F. & Batetta, B. 2013. Role of HDL in cholesteryl ester metabolism of lipopolysaccharide-activated P388D1 macrophages. *Journal of Lipid Research*, 54(11): 3158–3169.

Umehara, T., Kagawa, S., Tomida, A., Murase, T., Abe, Y., Shingu, K. & Ikematsu, K. 2020. Body temperature-dependent microRNA expression analysis in rats: rno-miR-374-5p regulates apoptosis in skeletal muscle cells via Mex3B under hypothermia. *Scientific Reports*, 10(1): 15432.

Valadi, H., Ekström, K., Bossios, A., Sjöstrand, M., Lee, J. & Lotvall, J. 2007. Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. *Nature Cell Biology*, 9(6): 654–659.

Van den Berg, A., Mols, J. & Han, J. 2008. RISC-target interaction: cleavage and translational suppression. *Biochim Biophys Acta*, 1779(11): 668–677.

Van der Pol, E., Böing, A., Harrison, P., Sturk, A. & Nieuwland, R. 2012. Classification, functions,

and clinical relevance of extracellular vesicles. *Pharmacological Reviews*, 64(3): 676–705.

Van Niekerk, A., Laubscher, R. & Laflamme, L. 2009. Demographic and circumstantial accounts of burn mortality in Cape Town, South Africa, 2001-2004: An observational register based study. *BioMed Central Public Health*, 9(1): 374.

Van Niekerk, A., Reimers, A. & Laflamme, L. 2006. Area characteristics and determinants of hospitalised childhood burn injury: A study in the city of Cape Town. *Public Health*, 120(2): 115–124.

Van Niekerk, A., Rode, H. & Laflamme, L. 2004. Incidence and patterns of childhood burn injuries in the Western Cape, South Africa. *Burns*, 30(4): 341–347.

Van Rooij, E., Quiat, D., Johnson, B.A., Sutherland, L.B., Qi, X., Richardson, J.A., Kelm, R.J. & Olson, E.N. 2009. A family of microRNAs encoded by myosin genes governs myosin expression and muscle performance. *Developmental Cell*, 17(5): 662–673.

Vandivier, R.W., Fadok, V.A., Hoffmann, P.R., Bratton, D.L., Penvari, C., Brown, K.K., Brain, J.D., Accurso, F.J. & Henson, P.M. 2002. Elastase-mediated phosphatidylserine receptor cleavage impairs apoptotic cell clearance in cystic fibrosis and bronchiectasis. *Journal of Clinical Investigation*, 109(5): 661–670.

Várallyay, É., Burgyán, J. & Havelda, Z. 2007. Detection of microRNAs by Northern blot analyses using LNA probes. *Methods*, 43(2): 140–145.

Várallyay, É., Burgyán, J. & Havelda, Z. 2008. MicroRNA detection by northern blotting using locked nucleic acid probes. *Nature Protocols*, 3(2): 190–196.

Vasudevan, S. & Steitz, J.A. 2007. AU-rich-element-mediated upregulation of translation by FXR1 and Argonaute 2. *Cell*, 128(6): 1105–1118.

Veeravagu, A., Yoon, B.C., Jiang, B., Carvalho, C.M., Rincon, F., Maltenfort, M., Jallo, J. & Ratliff, J.K. 2015. National trends in burn and inhalation injury in burn patients: Results of analysis of the nationwide inpatient sample database. *Journal of Burn Care & Research*, 36(2): 258–265.

Viart, V., Bergougnoux, A., Bonini, J., Varilh, J., Chiron, R., Tabary, O., Molinari, N., Claustres, M. & Taulan-Cadars, M. 2015. Transcription factors and miRNAs that regulate fetal to adult CFTR expression change are new targets for cystic fibrosis. *European Respiratory Journal*, 45(1): 116–128.

Vickers, K.C., Palmisano, B.T., Shoucri, B.M., Shamburek, R.D. & Remaley, A.T. 2011. MicroRNAs are transported in plasma and delivered to recipient cells by high-density lipoproteins. *Nature Cell Biology*, 13(4): 423–433.

Vickers, K.C. & Remaley, A.T. 2012. Lipid-based carriers of microRNAs and intercellular communication. *Current Opinion in Lipidology*, 23(2): 91–97.

Vico, P. & Papillon, J. 1992. Factors involved in burn mortality: A multivariate statistical approach based on discriminant analysis. *Burns*, 18(3): 212–215.

Vidotto, T., Melo, C.M., Castelli, E., Koti, M., dos Reis, R.B. & Squire, J.A. 2020. Emerging role of PTEN loss in evasion of the immune response to tumours. *British Journal of Cancer*, 122(12): 1732–1743.

Voelkerding, K. V, Dames, S.A. & Durtschi, J.D. 2009. Next-Generation Sequencing: From Basic Research to Diagnostics. *Clinical Chemistry*, 55(4): 641–658.

Vousden, K.H. 2005. p53 and PUMA: A Deadly Duo. *Science*, 309(5741): 1685–1686.

Wadey, K.S., Somos, A., Leyden, G., Blythe, H., Chan, J., Hutchinson, L., Poole, A., Frankow, A., Johnson, J.L. & George, S.J. 2023. Pro-inflammatory role of Wnt/β-catenin signaling in

endothelial dysfunction. *Frontiers in Cardiovascular Medicine*, 9: 1059124.

Wahid, F., Shehzad, A., Khan, T. & Young, Y. 2010. MicroRNAs : Synthesis , mechanism , function , and recent clinical trials. *Biochimica et Biophysica Acta*, 1803(11): 1231–1243.

Walker, P.F., Buehner, M.F., Wood, L.A., Boyer, N.L., Driscoll, I.R., Lundy, J.B., Cancio, L.C. & Chung, K.K. 2015. Diagnosis and management of inhalation injury : an updated review. *Critical Care*, 19: 351.

Waller, A.E., Marshall, S.W. & Langley, J.D. 1998. Adult thermal injuries in New Zealand resulting in death and hospitalization. *Burns*, 24(3): 245–251.

Walls, R., Olivier, G. & Eksteen, R. 2017. Informal settlement fires in South Africa: Fire engineering overview and full-scale tests on "shacks". *Fire Safety Journal*, 91: 997–1006.

Wang, B. & Xi, Y. 2013. Challenges for microRNA nicroarray data analysis. *Microarrays*, 2(2): 34–50.

Wang, B., Xu, J., Fu, P. & Ma, L. 2023a. MicroRNAs in septic acute kidney injury. *Burns & Trauma*, 11: tkad008.

Wang, C., Song, S., Zhang, Y., Ge, Y., Fang, X., Huang, T., Du, J. & Gao, J. 2015. Inhibition of the Rho/Rho kinase pathway prevents lipopolysaccharide-induced hyperalgesia and the release of TNF-α and IL-1β in the mouse spinal cord. *Scientific Reports*, 5(1): 14553.

Wang, E., Simard, M., Ouellet, N., Bergeron, Y., Beauchamp, D. & Bergeron, M.G. 2000. Modulation of cytokines and chemokines, limited pulmonary vascular bed permeability, and prevention of septicemia and death with ceftriaxone and interleukin-10 in Pneumococcal Pneumonia. *The Journal of Infectious Diseases*, 182(4): 1255–1259.

Wang, F., Kang, X., Li, Y., Lu, J., Liu, X. & Yan, H. 2024. Elucidating hepatocellular carcinoma progression: a novel prognostic miRNA–mRNA network and signature analysis. *Scientific Reports*, 14(1): 5042

Wang, F. & Mei, X. 2023. Expression level of serum miR-374a-5p in patients with acute pancreatitis and its effect on viability, apoptosis, and inflammatory factors of pancreatic acinar cells induced by cerulein. *The Kaohsiung Journal of Medical Sciences*, 39(6): 616–623.

Wang, K., Yuan, Y., Cho, J.-H., McClarty, S., Baxter, D. & Galas, D.J. 2012. Comparing the MicroRNA spectrum between serum and plasma. *PLoS One*, 7(7): e41561–e41561.

Wang, L., Huang, Z., Huang, W., Chen, X., Shan, P., Zhong, P., Khan, Z., Wang, J., Fang, Q., Liang, G. & Wang, Y. 2017. Inhibition of epidermal growth factor receptor attenuates atherosclerosis via decreasing inflammation and oxidative stress. *Scientific Reports*, 7(1): 45917.

Wang, Q., Xie, J.-F., Yao, T.-T., Wang, X.-X., Guo, Q.-W., Wang, L.-S., Yu, Y. & Xu, L.-C. 2023b. MicroRNA-30a-5p regulates cypermethrin-induced apoptosis of Sertoli cells by targeting KLF9 in vitro. *Reproductive Toxicology*, 119: 108414.

Wang, W., Liu, Z., Su, J., Chen, W.-S., Wang, X.-W., Bai, S.-X., Zhang, J.-Z. & Yu, S.-Q. 2016. Macrophage micro-RNA-155 promotes lipopolysaccharide-induced acute lung injury in mice and rats. *American Journal of Physiology*, 311(2): L494–L506.

Wang, X., Wang, K., Han, L., Zhang, A., Shi, Z., Zhang, K., Zhang, H., Yang, S., Pu, P., Shen, C., Yu, C. & Kang, C. 2013. PRDM1 is directly targeted by miR-30a-5p and modulates the Wnt/β-catenin pathway in a Dkk1-dependent manner during glioma growth. *Cancer Letters*, 331(2): 211–219.

Wang, Y., Li, Huan, Shi, Y., Wang, S., Xu, Y., Li, Hanyi & Liu, D. 2020. MiR-143-3p impacts on pulmonary inflammatory factors and cell apoptosis in mice with mycoplasmal pneumonia by

regulating TLR4/MyD88/NF-κB pathway. *Bioscience Reports*, 40(7): BSR20193419.

Wang, Y., Weng, T., Gou, D., Chen, Z., Chintagari, N.R. & Liu, L. 2007. Identification of rat lung-specific microRNAs by microRNA microarray: Valuable discoveries for the facilitation of lung research. *BioMed Central Genomics*, 8(1): 29.

Wang, Z., Fang, J. & Xiao, J. 2018. Correlation of the expression of inflammatory factors with expression of apoptosis-related genes Bax and Bcl-2, in burned rats. *Experimental and Therapeutic Medicine*, 17(3): 1790–1796.

Ware, L.B. & Matthay, M.A. 2001. Alveolar fluid clearance is impaired in the majority of patients with acute lung injury and the acute respiratory distress syndrome. *American Journal of Respiratory & Critical Care Medicine*, 163(6): 1376–1383.

Ware, L.B. & Matthay, M.A. 2000. The acute respiratory distress syndrome. *New England Journal of Medicine*, 342(18): 1334–1349.

Watkins, D.N., Peroni, D.J., Basclain, K.A., Garlepp, M.J. & Thompson, P.J. 1997. Expression and activity of nitric oxide synthases in human airway epithelium. *American Journal of Respiratory Cell & Molecular Biology*, 16(6): 629–639.

Weaver, L. 2009. Weaver LK. Clinical practice. Carbon monoxide poisoning. *The New England Journal of Medicine*, 360(12): 1217–1225.

Wei, B., Li, Z., Wang, L., Zhang, H. & Gou, W. 2024. MiR-200b-3p relieved inflammation in patients with heart failure by regulating ZEB1 expression. *Journal of Cardiothoracic Surgery*, 19(1): 271.

Wei, W., Yang, Y., Cai, J., Cui, K., Li, R. xian, Wang, H., Shang, X. & Wei, D. 2016. MiR-30a-5p suppresses tumor metastasis of human colorectal cancer by targeting ITGB3. *Cellular Physiology and Biochemistry*, 39(3): 1165–1176.

Weissman, R., Diamond, E.L., Haroche, J., Durham, B.H., Cohen, F., Buthorn, J., Amoura, Z., Emile, J.-F., Mazor, R.D., Shomron, N., Abdel-Wahab, O.I., Shpilberg, O. & Hershkovitz-Rokah, O. 2022. MicroRNA-15a-5p acts as a tumor suppressor in histiocytosis by mediating CXCL10-ERK-LIN28a-let-7 axis. *Leukemia*, 36(4): 1139–1149.

Wetterslev, J., Meyhoff, C.S., Jørgensen, L.N., Gluud, C., Lindschou, J. & Rasmussen, L.S. 2015. The effects of high perioperative inspiratory oxygen fraction for adult surgical patients. *Cochrane Database of Systematic Reviews*, 2015(6): CD008884.

Wetzker, R. & Böhmer, F.-D. 2003. Transactivation joins multiple tracks to the ERK/MAPK cascade. *Nature Reviews Molecular Cell Biology*, 4(8): 651–657.

Weyker, P.D., Webb, C.A.J., Kiamanesh, D. & Flynn, B.C. 2013. Lung ischemia reperfusion injury: A bench-to-bedside review. *Seminars in Cardiothoracic & Vascular Anesthesia*, 17(1): 28–43.

Whitehead, C.L., Teh, W.T., Walker, S.P., Leung, C., Larmour, L. & Tong, S. 2013. Circulating MicroRNAs in maternal blood as potential biomarkers for fetal hypoxia in-utero. *PLoS One*, 8(11): e78487–e78487.

Whitelock-Jones, L., Bass, D.H., Millar, A.J.W. & Rode, H. 1999. Inhalation burns in children. *Pediatric Surgery International*, 15(1): 50–55.

Wieckowski, E. & Whiteside, T.L. 2006. Human tumor-derived vs dendritic cell-derived exosomes have distinct biologic roles and molecular profiles. *Immunologic Research*, 36(1): 247–254.

Wightman, B., Ha, I. & Ruvkun, G. 1993. Posttranscriptional regulation of the heterochronic gene lin-14 by lin-4 mediates temporal pattern formation by C. elegans. *Cell*, 75: 855–862.

Wittram, C. & Kenny, J.B. 1994. The admission chest radiograph after acute inhalation injury and burns. *The British Journal of Radiology*, 67(800): 751–754.

Wold, H. 1985. Partial least squares. In S. Kotz & N. L. Johnson, eds. *Encyclopedia of Statistical Sciences*. New York: Wiley: 581–591.

Woods, J., Quinlan, C. & Shelley, O. 2016. Predicting Mortality in Severe Burns—What Is the Score? *Plastic & Reconstructive Surgery*, 4(1): e606.

Woodson, L.C. 2009. Diagnosis and grading of inhalation injury. *Journal of Burn Care & Research*, 30(1): 143–145.

World Health Organization. 2018a. *Burns Key Facts*. https://www.who.int/news-room/fact-sheets/detail/burns [21 May 2021].

World Health Organization. 2008. *Global Burden of Disease 2004 Summary Tables*.. https://www.who.int/healthinfo/global_burden_disease/estimates_regional /en/index.html [20 July 2019].

World Health Organization. 2018b. *Global Health Estimates 2016: Disease burden by Cause, Age, Sex, by Country and by Region 2000-2016*. https://www.who.int/healthinfo/global_burden_disease/GHE2016_DALY_WHOReg_2000_2016_.xls?ua=1 [20 August 2019].

Wu, K.K., Xu, X., Wu, M., Li, X., Hoque, M., Li, G.H.Y., Lian, Q., Long, K., Zhou, T., Piao, H., Xu, A., Hui, H.X. & Cheng, K.K. 2024. MDM2 induces pro-inflammatory and glycolytic responses in M1 macrophages by integrating iNOS-nitric oxide and HIF-1α pathways in mice. *Nature Communications*, 15(1): 8624.

Wu, L., Chen, Y., Chen, Y., Yang, W., Han, Y., Lu, L., Yang, K. & Cao, J. 2019. Effect of HIF-1α/miR-10b-5p/PTEN on hypoxia-induced cardiomyocyte apoptosis. *Journal of the American Heart Association*, 8(18): e011948.

Wu, L., Zhang, R., Lin, S., Lin, M. & Wang, J. 2021. Silencing CDK6-AS1 inhibits LPS-induced inflammatory damage in HK-2 cells. *Open Medicine*, 16(1): 1256–1264.

Wu, X., Zhang, Y., Wang, P., Li, X., Song, Z., Wei, C., Zhang, Q., Luo, B., Liu, Z., Yang, Y., Ren, Z. & Liu, H. 2023. Clinical and preclinical evaluation of miR-144-5p as a key target for major depressive disorder. *CNS Neuroscience & Therapeutic*s, 29(11): 3598–3611.

Wu, X.-B., Wang, M.-Y., Zhu, H.-Y., Tang, S.-Q., You, Y.-D. & Xie, Y.-Q. 2014. Overexpression of microRNA-21 and microRNA-126 in the patients of bronchial asthma. *International Journal of Clinical & Experimental Medicine*, 7(5): 1307–12.

Xi, Y., Nakajima, G., Gavin, E., Morris, C.G., Kudo, K., Hayashi, K. & Ju, J. 2007. Systematic analysis of microRNA expression of RNA extracted from fresh frozen and formalin-fixed paraffin-embedded samples. *RNA*, 13(10): 1668–1674.

Xia, M. & Land, H. 2007. Tumor suppressor p53 restricts Ras stimulation of RhoA and cancer cell motility. *Nature Structural & Molecular Biology*, 14(3): 215–223.

Xiao, P., Sun, S., Cao, J., Wang, J., Li, H., Hou, S., Ding, H., Liu, Z., Fang, Y., Bai, S., Qin, X., Yu, F., Liu, J., Wang, X., Lv, Q. & Fan, H. 2018. Expression profile of microRNAs in bronchoalveolar lavage fluid of rats as predictors for smoke inhalation injury. *Burns*, 44(8): 2042–2050.

Xiao, Q., Werner, J., Venkatachalam, N., Boonekamp, K.E., Ebert, M.P. & Zhan, T. 2022. Cross-talk between p53 and Wnt signaling in cancer. *Biomolecules*, 12(3): 453.

Xie, L., Mouillet, J.-F., Chu, T., Parks, W.T., Sadovsky, E., Knöfler, M. & Sadovsky, Y. 2014. C19MC microRNAs regulate the migration of human trophoblasts. *Endocrinology*, 155(12): 4975–4985.

Xie, Z., Bailey, A., Kuleshov, M. V., Clarke, D.J.B., Evangelista, J.E., Jenkins, S.L., Lachmann, A., Wojciechowicz, M.L., Kropiwnicki, E., Jagodnik, K.M., Jeon, M. & Ma'ayan, A. 2021. Gene set knowledge discovery with Enrichr. *Current Protocols*, 1(3): e90.

Xu, C., Zhou, G., Wang, X., Zhang, B., Zhao, T. & Wu, L. 2022. Correlation analysis of serum miR-21 and miR-210 with hs-CRP, TNF-α, IL-6, and ICAM-1 in patients with sepsis after burns. *Burns*, 48(3): 633–638.

Xu, J. & Lin, N. 2024. HOXD10 regulates intestinal permeability and inhibits inflammation of dextran sulfate sodium-induced ulcerative colitis through the inactivation of the Rho/ROCK/MMPs axis. *Open Medicine*, 19(1): 20230844.

Xu, X., Steere, R.R., Fedorchuk, C.A., Pang, J., Lee, J.-Y., Lim, J.H., Xu, H., Pan, Z.K., Maggirwar, S.B. & Li, J.-D. 2011. Activation of Epidermal Growth Factor Receptor Is Required for NTHi-Induced NF-κB-Dependent Inflammation. *PLoS One*, 6(11): e28216.

Xue, J., Liu, J., Xu, B., Yu, J., Zhang, A., Qin, L., Liu, C. & Yang, Y. 2021. MiR-21-5p inhibits inflammation injuries in LPS-treated H9c2 cells by regulating PDCD4. *American Journal of Translational Research*, 13(10): 11450–11460.

Yamamoto, R., Shibusawa, T., Aikawa, N. & Sasaki, J. 2020. Modified abbreviated burn severity index as a predictor of in-hospital mortality in patients with inhalation injury: development and validation using independent cohorts. *Surgery Today*, 51(2): 242–249

Yamamoto, Y. & Gaynor, R.B. 2001. Therapeutic potential of inhibition of the NF-κB pathway in the treatment of inflammation and cancer. *Journal of Clinical Investigation*, 107(2): 135–142.

Yamaya, Y., Bogaard, H.J., Wagner, P.D., Niizeki, K. & Hopkins, S.R. 2002. Validity of pulse oximetry during maximal exercise in normoxia, hypoxia, and hyperoxia. *Journal of Applied Physiology*, 92(1): 162–168.

Yamaza, T., Masuda, K.F., Tsukiyama, Y., Nishijima, K., Murakami, R., Kido, M.A., Koyano, K. & Tanaka, T. 2003. NF-κB Activation and iNOS expression in the synovial membrane of rat temporomandibular joints after induced synovitis. *Journal of Dental Research*, 82(3): 183–188.

Yan, X., Liu, Y., Kong, X., Ji, J., Zhu, H., Zhang, Zexu, Fu, T., Yang, J., Zhang, Zhongyuan, Liu, F. & Gu, Z. 2019a. MicroRNA-21-5p are involved in apoptosis and invasion of fibroblast-like synoviocytes through PTEN/PI3K/AKT signal. *Cytotechnology*, 71(1): 317–328.

Yan, Y., Lu, K., Ye, T. & Zhang, Z. 2019b. MicroRNA-223 attenuates LPS-induced inflammation in an acute lung injury model via the NLRP3 inflammasome and TLR4/NF-κB signaling pathway via RHOB. *International Journal of Molecular Medicine*, 43(3): 1467–1477.

Yang, H., Lu, Z., Huo, C., Chen, Y., Cao, H., Xie, P., Zhou, H., Liu, D., Liu, J. & Yu, L. 2019. Liang-Ge-San, a classic traditional chinese medicine formula, attenuates lipopolysaccharide-induced acute lung injury through up-regulating miR-21. *Frontiers in Pharmacology*, 10: 1332.

Yang, H.T., Yim, H., Cho, Y.S., Kim, D., Hur, J., Chun, W., Kim, J.H., Jung, S.Y., Kim, B.C. & Lee, J.J. 2011. Investigation of relationship between inhalation injury assessment and prognosis in burn patients. *Journal of the Korean Surgical Society*, 81(1): 1–9.

Yang, R., Guo, P., Ma, Z., Chang, C., Meng, Q., Gao, Y., Khan, I., Wang, X. & Cui, Z. 2020. Effects of simvastatin on iNOS and caspase-3 levels and oxidative stress following smoke inhalation injury. *Molecular Medicine Reports*, 22(4): 3405–3417.

Yang, S., Zou, X., Yang, H., Li, J., Zhang, A., Zhang, L., Li, C., Zhu, L. & Ma, Z. 2022. Identification of enhancer RNA CDK6-AS1 as a potential novel prognostic biomarker in gastric cancer. *Frontiers in Genetics*, 13: 854211.

Yang, Z., Wang, J., Pan, Z. & Zhang, Y. 2018. MiR-143-3p regulates cell proliferation and apoptosis

by targeting IGF1R and IGFBP5 and regulating the Ras/p38 MAPK signaling pathway in rheumatoid arthritis. *Experimental & Therapeutic Medicine*, 15(4): 3781–3790.

Yasuda, K., Henmi, H., Yamamoto, Y., Mashiko, K., Ohtomo, Y. & Ohtuka, T. 1986. Nutritional management and assessment on extensively burned patients (in Japanese). *Japanese Journal of Burn Injuries*, 11: 134–138.

Ye, J., Xu, M., Tian, X., Cai, S. & Zeng, S. 2019. Research advances in the detection of miRNA. *Journal of Pharmaceutical Analysis*, 9(4): 217–226.

Yeung, M.L., Bennasser, Y., Le, S.Y. & Jeang, K.T. 2005. siRNA, miRNA and HIV: Promises and challenges. *Cell Research*, 15(11–12): 935–946.

Ying, S.-Y., Chang, D.C. & Lin, S.-L. 2008. The microRNA (miRNA): Overview of the RNA genes that modulate gene function. *Molecular Biotechnology*, 38(3): 257–268.

Yook, S., Oltvai, Z.N. & Barabási, A. 2004. Functional and topological characterization of protein interaction networks. *Proteomics*, 4(4): 928–942.

You, H., Pellegrini, M., Tsuchihara, K., Yamamoto, K., Hacker, G., Erlacher, M., Villunger, A. & Mak, T.W. 2006. FOXO3a-dependent regulation of Puma in response to cytokine/growth factor withdrawal. *The Journal of Experimental Medicine*, 203(7): 1657–1663.

You, K., Yang, H.-T., Kym, D., Yoon, J., Haejun, Y., Cho, Y.-S., Hur, J., Chun, W. & Kim, J.-H. 2014. Inhalation injury in burn patients: Establishing the link between diagnosis and prognosis. *Burns*, 40(8): 1470–1475.

Young, C.J. & Moss, J. 1989. Smoke inhalation: Diagnosis and treatment. *Journal of Clinical Anesthesia*, 1(5): 377–386.

Yu, J., Wang, Z., Kinzler, K.W., Vogelstein, B. & Zhang, L. 2003. PUMA mediates the apoptotic response to p53 in colorectal cancer cells. *Proceedings of the National Academy of Sciences*, 100(4): 1931–1936.

Yu, Y., Li, X., Liu, L., Chai, J., Haijun, Z., Chu, W., Yin, H., Ma, L., Duan, H. & Xiao, M. 2016. miR-628 promotes burn-induced skeletal muscle atrophy via targeting IRS1. *International Journal of Biological Sciences*, 12(10): 1213–1224.

Zeng, F., He, J., Jin, X., Liao, Q., Chen, Z., Peng, H. & Zhou, Y. 2022. FRA-1: A key factor regulating signal transduction of tumor cells and a potential target molecule for tumor therapy. *Biomedicine & Pharmacotherapy*, 150: 113037.

Zeng, H., Zhou, Y., Liu, Z. & Liu, W. 2024. MiR-21-5p modulates LPS-induced acute injury in alveolar epithelial cells by targeting SLC16A10. *Scientific Reports*, 14(1): 11160.

Zhai, H., Huang, L., Gong, Y., Liu, Y., Wang, Y., Liu, B., Li, X., Peng, C. & Li, T. 2022. Human plasma transcriptome implicates dysregulated S100A12 expression: A strong, early-stage prognostic factor in ST-segment elevated myocardial infarction: Bioinformatics analysis and experimental verification. *Frontiers in Cardiovascular Medicine*, 9: 87443.

Zhan, L., Mu, Z., Jiang, H., Zhang, S., Pang, Y., Jin, H., Chen, J., Jia, C. & Guo, H. 2023. MiR-21-5p protects against ischemic stroke by targeting IL-6R. Annals of Translational Medicine, 11(2): 101–101.

Zhan, Q., Cui, P., Pan, Y., Dai, Z., Gong, Z., Xin, H., Tang, F., Deng, C., Huang, G., Zhu, F. & Tong, Y. 2019. Investigating the potential to assess severe lung inhalation injuries using computed tomography. *Burns*, 45(2): 310–316.

Zhang, C. 2008. MicroRNomics: a newly emerging approach for disease biology. *Physiological Genomics*, 33(2): 139–147.

Zhang, D., Chang, Y., Han, S., Yang, L., Hu, Q., Yu, Y., Liu, L. & Chai, J. 2018. The microRNA expression profile in rat lung tissue early after burn injury. *Turkish Journal of Trauma & Emergency Surgery*, 24(3): 191–198.

Zhang, F., Cheng, N., Du, J., Zhang, H. & Zhang, C. 2021a. MicroRNA-200b-3p promotes endothelial cell apoptosis by targeting HDAC4 in atherosclerosis. BMC Cardiovascular Disorders, 21(1): 172.

Zhang, F., Gou, Z., Zhou, Y., Huang, L., Shao, C., Wang, M., Wu, C. & Lu, L. 2022. MicroRNA-21-5p agomir inhibits apoptosis of oligodendrocyte precursor cell and attenuates white matter injury in neonatal rats. *Brain Research Bulletin*, 189: 139–150.

Zhang, H., Wei, W., Zhao, M., Ma, L., Jiang, X., Pei, H., Cao, Y. & Li, H. 2021b. Interaction between Aβ and Tau in the Pathogenesis of Alzheimer's Disease. *International Journal of Biological Sciences*, 17(9): 2181–2192.

Zhang, J., Li, S., Li, L., Li, M., Guo, C., Yao, J. & Mi, S. 2015. Exosome and exosomal MicroRNA: Trafficking, sorting, and function. *Genomics, Proteomics & Bioinformatics*, 13(1): 17–24.

Zhang, K., Cheng, M., Xu, J., Chen, L., Li, J., Li, Q., Xie, X. & Wang, Q. 2020a. MiR-711 and miR-183-3p as potential markers for vital reaction of burned skin. *Forensic Sciences Research*, 7(3): 503–509.

Zhang, K., Zhong, W., Li, W.-P., Chen, Z.-J. & Zhang, C. 2017a. MiR-15a-5p levels correlate with poor ovarian response in human follicular fluid. *Reproduction*, 154(4): 483–496.

Zhang, N., Yang, L., Wen, J. & Cui, H. 2023. MiR-200b-3p antagomir inhibits neuronal apoptosis in oxygen-glucose deprivation (OGD) model through regulating β-TrCP. *Brain Research*, 1800: 148192.

Zhang, S., Li, Y., Wu, Y., Shi, K., Bing, L. & Hao, J. 2012. Wnt/β-Catenin signaling pathway upregulates c-Myc expression to promote cell proliferation of P19 teratocarcinoma cells. *The Anatomical Record*, 295(12): 2104–2113.

Zhang, W-L, Chi, C., Meng, X. & Liang, S. 2019a. MiRNA-15a-5p facilitates the bone marrow stem cell apoptosis of femoral head necrosis through the Wnt/β-catenin/PPARγ signaling pathway. *Molecular Medicine Reports*, 19(6): 4779–4787.

Zhang, W, Li, Y., Xi, X., Zhu, G., Wang, S., Liu, Y. & Song, M. 2019b. MicroRNA-15a-5p induces pulmonary artery smooth muscle cell apoptosis in a pulmonary arterial hypertension model via the VEGF/p38/MMP-2 signaling pathway. I*nternational Journal of Molecular Medicine*, 45(2): 461–474.

Zhang, Y., Huang, H., Liu, W., Liu, S., Wang, X.Y., Diao, Z.L., Zhang, A.H., Guo, W., Han, X., Dong, X. & Katilov, O. 2021c. Endothelial progenitor cells-derived exosomal microRNA-21-5p alleviates sepsis-induced acute kidney injury by inhibiting RUNX1 expression. *Cell Death & Disease*, 12(4): 335.

Zhang, Y, Saradna, A., Ratan, R., Ke, X., Tu, W., Do, D.C., Hu, C. & Gao, P. 2020b. RhoA/Rho-kinases in asthma: from pathogenesis to therapeutic targets. *Clinical & Translational Immunology*, 9(5): e1134.

Zhang, Y, Xie, Y., Zhang, L. & Zhao, H. 2020c. MicroRNA-155 participates in smoke-inhalation-induced acute lung injury through inhibition of SOCS-1. *Molecules*, 25(5): 1022.

Zhang, Y., Yin, B., Shu, B., Liu, Z., Ding, H. & Jia, C. 2017b. Differential expression of microRNA let-7b-5p regulates burn-induced hyperglycemia. *Oncotarget*, 8(42): 72886–72892.

Zhao, Y., Pan, S., Li, Y. & Wu, X. 2022. Exosomal miR-143-3p derived from follicular fluid promotes granulosa cell apoptosis by targeting BMPR1A in polycystic ovary syndrome.

Scientific Reports, 12(1): 4359.

Zhou, D., Zhou, T., Tang, S., Li, Q., Li, W., Gan, G., Li, M. & Chen, Q. 2024. Network pharmacology combined with Mendelian randomization analysis to identify the key targets of renin-angiotensin-aldosterone system inhibitors in the treatment of diabetic nephropathy. *Frontiers in Endocrinology*, 15: 1354950.

Zhou, F., Liu, Z., Cai, H., Miao, Z., Wei, F. & Song, C. 2020. Role of microRNA-15a-5p/TNFAIP3-interacting protein 2 axis in acute lung injury induced by traumatic hemorrhagic shock. *Experimental and Therapeutic Medicine*, 20(5): 2.

Zhou, J., Zhang, X., Liang, P., Ren, L., Zeng, J., Zhang, M., Zhang, P. & Huang, X. 2016. Protective role of microRNA-29a in denatured dermis and skin fibroblast cells after thermal injury. *Biology Open*, 5(3): 211–219.

Zhou, Q., Li, M., Wang, Xiaoyan, Li, Q., Wang, T., Zhu, Q., Zhou, X., Wang, Xin, Gao, X. & Li, X. 2012. Immune-related microRNAs are abundant in breast milk exosomes. *International Journal of Biological Sciences*, 8(1): 118–123.

Zhou, R., Zhang, Q., Zhang, Y., Fu, S. & Wang, C. 2015. Aberrant miR-21 and miR-200b expression and its pro-fibrotic potential in hypertrophic scars. *Experimental Cell Research*, 339(2): 360–366.

Zhu, H.-Y., Li, C., Bai, W.-D., Su, L.-L., Liu, J.-Q., Li, Y., Shi, J.-H., Cai, W.-X., Bai, X.-Z., Jia, Y.-H., Zhao, B., Wu, X., Li, J. & Hu, D.-H. 2014. MicroRNA-21 regulates hTERT via PTEN in hypertrophic scar fibroblasts. *PloS One*, 9(5): e97114.

Zhu, Q.-J., Wang, J., Li, Y., Bai, Z.-J., Guo, X.-B. & Pan, T. 2023. PRKCA Promotes Mitophagy through the miR-15a-5p/PDK4 Axis to Relieve Sepsis-Induced Acute Lung Injury. *Infection & Immunit*y, 91(1): e0046522.

Zhu, W., Xu, J., Zhang, M., Zhu, T., Zhang, Y. & Sun, K. 2018. MicroRNA-21 inhibits lipopolysaccharide-induced acute lung injury by targeting nuclear factor-κB. *Experimental & Therapeutic Medicine*, 16: 4616–4622.

Zhu, W., Zhou, Z., Wu, C., Huang, Z., Zhao, R., Wang, X., Luo, L., Liu, Y., Zhong, W., Zhao, Z., Ai, G., Zhong, J., Liu, S., Liu, W., Pang, X., Sun, Y. & Zeng, G. 2024. MiR-148b-5p regulates hypercalciuria and calcium-containing nephrolithiasis. *Cellular & Molecular Life Sciences*, 81(1): 369.

Zhu, X., Gerstein, M. & Snyder, M. 2007. Getting connected: analysis and principles of biological networks. *Genes & Development*, 21(9): 1010–1024.

Zhuang, W., Ye, T., Wang, W., Song, W. & Tan, T. 2023. CTNNB1 in neurodevelopmental disorders. *Frontiers in Psychiatry*, 14: 1143328.

APPENDIX A: Epidemiology and mortality

Table 9: Frequencies and distributions of socio-demographic factors for the study population (n=59)

	Total samples, N (%)	Fold change [a]	P-value	Mortality, N (%)	Fold change	Mortality rate (%)
	59 (100)			15 (25.4)		25.4
Gender						
Male	38 (64.4)	1.8	0.000	9 (60)	1.5	23.7
Female	21 (35.6)			6 (40)		28.6
Age (years)						
13-20	8 (13.6)	4.8 – 6.3	≤0.001	4 (26.7)	1.5 – 3	50
21-39	38 (64.4)			6 (40)		15.8
40-49	7 (11.9)			3 (20)		42.9
≥ 50	6 (10.2)			2 (13.3)		33.3
Day of the week [b]						
Weekdays	37 (62.7)	1.7	0.000	8 (53.3)	1.1	21.6
Weekend days	22 (37.3)			7 (46.7)		31.8
Seasonal variation [c]						
Colder seasons	36 (61.1)	1.6	0.000	10 (66.7)	2	27.8
Warmer seasons	23 (38.9)			5 (33.3)		21.7
Referral setting (district)						
City of Cape Town	50 (84.7)	5.6	0.000	10 (66.7)	2	20
Other [d]	9 (15.3)			5 (33.3)		55.6
Level of referral						
Hospital	46 (78)	3.5	0.000	11 (73.3)	2.8	23.9
CHC/clinic [e]	13 (22)			4 (26.7)		30.8

[a] Fold change between more than two groups are affiliated with the highest frequency within the variable
[b] Days of the week: weekdays - Monday to Friday, weekend days - Saturday and Sunday
[c] Seasonal variation: Colder seasons – Autumn (March to May) and Winter (June to August), warmer seasons – Spring (September to November) and Summer (December to February)
[d] Other: districts outside of City of Cape Town that included Cape Winelands, Eden/Garden route, Overberg and West Coast. Zero patients were transferred from the Central Karoo district
[e] CHC: community health centre

Table 10: Frequencies and distributions of injury characteristics for the study population (n=59)

	Total samples, N (%)	Fold change [a]	P-value	Mortality, N (%)	Fold change	Mortality rate (%)
	59 (100)			15 (25.4)		25.4
Aetiology						
Fire	44 (74.6)	2.9	0.000	14 (93.3)	14	31.8
Other [b]	15 (25.4)			1 (6.7)		6.7
% Total Body Surface Area						
≤ 40%	52 (88.1)	7.4	0.000	8 (53.3)	1.1	15.4
> 40%	7 (11.9)			7 (46.7)		100
Inhalation injury [c]						
Mild (0-4 days total ventilation)	23 (39)	1.6	0.000	1 (6.7)	14	4.3
Severe (≥5 days total ventilation)	36 (61)			14 (93.3)		38.9
Phenotypical characteristics						
Singed nasal hairs	39 (66.1)	1.3 – 2.5	0.000	9 (60)	1.6 – 7	23.1
Soot around/in mouth	40 (67.8)			7 (46.7)		17.5
Hoarseness	21 (35.6)			2 (13.3)		9.5
Facial burns	52 (88.1)			14 (93.3)		26.9
Complications [d]						
Yes	25 (42.4)	1.4	0.000	15 (100)	15	60
No	34 (57.6)			0 (0)		0
ABSI scores [e]						
Moderate (4-5)	8 (13.6)	1.2 – 7.3	≤0.020	0 (0)	1.5 - 3	0
Moderately severe (6-7)	18 (30.5)			2 (13.3)		11.1
Serious (8-9)	22 (37.3)			4 (26.7)		18.1
Severe (10-11)	8 (13.6)			6 (40)		75
Maximum (12-13)	3 (5.1)			3 (20)		100

[a] Fold change between more than two groups are affiliated with the highest frequency within the variable
[b] Other (n): Steam/hot water (n=2), gas explosion (n=3), paraffin (n=3), electrical (n=4), petrol (n=1), chemical (n=1), assault/necklacing (n=1)
[c] Inhalation injury: Mild represented by 0-4 days total ventilation, severe represented by >5 days total ventilation
[d] Complications: Septic shock, septicemia, Acute Respiratory Distress Syndrome, ventilator associated pneumonia, and aspiration pneumonia
[e] ABSI: Abbreviated burn severity index

Table 11: Frequencies and distributions of clinical parameters for the study population (n=59)

	Total samples, N (%)	Fold change [a]	P-value	Mortality, N (%)	Fold change	Mortality rate (%)
	59 (100)			15 (25.4)		25.4
BICU LOS [b]						
0-9	30 (50.8)	1	0.000	3 (20)	4	10
≥ 10	29 (49.2)			12 (80)		41.4
Ventilation prior to admission						
Yes	51 (86.4)	6.4	0.000	14 (93.3)	14	27.4
No	8 (13.6)			1 (6.7)		12.5
Arterial blood gas analysis						
pH						
< 7.35 (acidosis)	24 (40.7)			9 (60)		42.9
7.35-7.45 (normal)	28 (47.5)	1.3 – 4.1	≤0.011	4 (26.7)	2.3 – 4.5	14.3
> 7.45 (alkalosis)	7 (11.9)			2 (13.3)		28.6
PaO_2 (kPa) [c]						
< 10.5 (hypoxia)	9 (15.3)			3 (20)		33.3
10.5-13.5 (normal)	9 (15.3)	4.6	0.000	1 (6.7)	3.7 – 11	11.1
> 13.5 (hyperoxia)	41 (69.5)			11 (73.3)		26.8
PCO_2 (kPa) [d]						
< 4.7	13 (22)			4 (26.7)		30.8
4.7-6.0 (normal)	29 (45.2)	1.9 – 2.3	0.000	7 (46.7)	1.8	24.1
> 6.0	17 (28.8)			4 (26.7)		23.5
SATS (%)						
< 95	7 (11.9)	7.4	0.000	2 (13.3)	6.5	28.6
95-100 (normal)	52 (88.1)			13 (86.7)		25
Lactate (mmol/L)						
< 2.0 (normal)	34 (57.6)	1.4	≤0.000	6 (40)	1.5	17.6
≥2.0 (excess)	25 (42.4)			9 (60)		36.0
Base excess (mmol/L)						
< -4	11 (18.6)			4 (26,7)		36.4
-4 to +2 (normal)	41 (69.5)	3.7 – 5.9	0.000	9 (60)	2.3 – 4.5	22
> 2	7 (11.9)			2 (13.3)		28.6

[a] Fold change between more than two groups are affiliated with the highest frequency within the variable
[b] BICU LOS: burns intensive care unit length of stay
[c] PaO_2: partial pressure of arterial oxygen
[d] PCO_2: partial pressure of arterial carbon dioxide

Table 12: PLS regression incorporating ABSI sub-groups for the selective inclusion in mortality prediction model.

			Predicted variances of latent factors	
	1	**2**	**3**	**4**
***X*-variance**	*0.232*	0.234	0.242	0.291
***Y*-variance**	*0.413*	0.002	5.859E-6	4.809E-8
Adjusted R-square (R^2)	*0.403*	0.394	0.382	0.371
			Latent factors VIP values per variable	
Moderate (4-5) score	0.748	0.750	0.750	0.750
Moderately severe (6-17) score	0.705	0.705	0.705	0.705
Serious (8-9) score	0.415	0.414	0.414	0.414
Severe (10-11) score	*1.459*	1.457	1.457	1.457
Maximum (12-13) score	*1.282*	1.282	1.282	1.282

VIP: variable importance in prediction, PLS: partial least squares, ABSI: abbreviated burn severity index.

APPENDIX B: Epidemiology, mortality and inhalation injury degree

Table 13: Frequencies and distributions of socio-demographic factors and mild (n=23) and severe (n=36) inhalation injury for the study population (n=59)

	Mild Inhalation injury (0-4 total days ventilated)					**Severe Inhalation injury** (≥ 5 total days ventilated)				
	Total samples, N (%)	**Fold change [a]**	**Mortality, N (%)**	**Fold change**	**Mortality rate (%)**	**Total samples, N (%)**	**Fold change**	**Mortality, N (%)**	**Fold change**	**Mortality rate (%)**
	23 (39)		1 (6.7)		4.3	36 (61)		14 (93.3)		38.9
Gender										
Male	12 (52.2)	1.1	0 (0)	N/A	0	26 (72.2)	2.6	9 (64.3)	1.8	34.6
Female	11 (47.8)		1 (100)		9.1	10 (27.7)		5 (35.7)		50
Age (years)										
13-20	3 (13)	3.75 – 15	1 (100)	N/A	33.3	5 (13.9)	3.8 – 11.5	3 (21.4)	2 – 3	60
21-39	15 (65.2)		0 (0)		0	23 (63.9)		6 (42.9)		26.1
40-49	1 (4.3)		0 (0)		0	6 (16.7)		3 (21.4)		50
≥ 50	4 (17.4)		0 (0)		0	2 (5.6)		2 (14.3)		100
Day of the week [b]										
Weekdays	15 (65.2)	1.9	1 (100)	N/A	6.7	22 (61.1)	1.6	7 (50)	1	31.8
Weekend days	8 (34.8)		0 (0)		0	14 (38.9)		7 (50)		50
Seasonal variation [c]										
Colder seasons	13 (56.5)	1.3	0 (0)	N/A	0	23 (63.9)	1.8	10 (71.4)	2.2	43.5
Warmer seasons	10 (43.5)		1 (100)		10	13 (36.1)		4 (28.6)		30.8
Referral setting (district)										
City of Cape Town	23 (100)	23	1 (100)	N/A	4.3	28 (77.8)	3.5	9 (64.3)	1.8	32.1
Other [d]	0 (0)		0 (0)			8 (22.2)		5 (35.7)		62.5
Level of referring hospital										
Hospital	19 (82.6)	4.8	0 (0)	N/A	0	27 (75)	3	11 (78.6)	3.7	40.7
CHC/clinic	4 (17.4)		1 (100)		25	9 (25)		3 (21.4)		33.3
Days between injury & admission										
0-2	21 (91.3)	10.5	1 (100)	N/A	4.8	30 (83.3)	7.5 – 15	11 (78.6)	5.5 – 11	36.7
3-5	2 (8.7)		0 (0)		0	4 (11.1)		1 (7.1)		25
> 5	0 (0)		0 (0)		0	2 (5.6)		2 (14.3)		100

[a] Fold change between more than two groups are affiliated with the highest frequency within the variable
[b] Days of the week: weekdays - Monday to Friday, weekend days - Saturday and Sunday
[c] Seasonal variation: Colder seasons – Autumn (March to May) and Winter (June to August), warmer seasons – Spring (September to November) and Summer (December to February)
[d] Other: districts outside of City of Cape Town that included Cape Winelands, Eden/Garden route, Overberg and West Coast. Zero patients were transferred from the Central Karoo district
[e] CHC: community health centre

Table 14: Frequencies and distributions of injury characteristics and mild (n=23) and severe (n=36) inhalation injury for the study population (n=59)

	Mild Inhalation injury (0-4 total days ventilated)					**Severe Inhalation injury** (≥ 5 total days ventilated)				
	Total samples, N (%)	**Fold change [a]**	**Mortality, N (%)**	**Fold change**	**Mortality rate (%)**	**Total samples, N (%)**	**Fold change**	**Mortality, N (%)**	**Fold change**	**Mortality rate (%)**
	23 (39)		1 (7.7)			36 (61)		14 (93.3)		
Aetiology										
Fire	18 (78.3)	3.6	1 (100)	N/A	5.6	26 (72.2)	2.6	13 (92.9)	13	50
Other [b]	5 (21.7)		0 (0)		0	10 (27.8)		1 (7.1)		10
% Total Body Surface Area										
≤ 40%	23 (100)	23	1 (100)	N/A	4.3	29 (80.6)	4.1	7 (50)	1	24.1
> 40%	0 (0)		0 (0)		0	7 (19.4)		7 (50)		100
Phenotypical characteristics										
Singed nasal hairs	15 (65.2)	1.2 – 1.6	0 (0)	N/A	0	24 (66.7)	1.4 – 3.7	8 (57.1)	1.6 – 6.5	33.3
Soot around/in mouth	16 (69.6)		0 (0)		0	24 (66.7)		8 (57.1)		33.3
Hoarseness	12 (52.2)		0 (0)		0	9 (25)		2 (14.3)		22.2
Facial burns	19 (82.6)		1 (100)		5.3	33 (91.7)		13 (92.9)		39.4
Complications [c]										
Yes	2 (8.7)	10.5	1 (100)	N/A	50	23 (63.9)	1.8	14 (100)	N/A	60.1
No	21 (91.3)		0 (0)		0	13 (36.1)		0 (0)		0
ABSI scores [d]										
Moderate (4-5)	5 (21.7)	1.1 – 9	0 (0)	N/A	0	3 (8.3)	1.6 – 4.7	0 (0)	1-5 – 6	0
Moderately severe (6-7)	9 (39.1)		1 (100)		11.1	9 (25)		1 (7.1)		11.1
Serious (8-9)	8 (34.8)		0 (0)		0	14 (38.9)		4 (28.6)		28.6
Severe (10-11)	1 (4.3)		0 (0)		0	7 (19.4)		6 (42.9)		85.7
Maximum (12-13)	0 (0)		0 (0)		0	3 (8.3)		3 (21.4)		100

[a] Fold change between more than two groups are affiliated with the highest frequency within the variable

[b] Other (n): Steam/hot water (n=2), gas explosion (n=3), paraffin (n=3), electrical (n=4), petrol (n=1), chemical (n=1), assault/necklacing (n=1)

[c] Complications: Septic shock, septicemia, Acute Respiratory Distress Syndrome, ventilator associated pneumonia, and aspiration pneumonia

[d] ABSI: Abbreviated burn severity index

Table 15: Frequencies and distributions of clinical parameters and mild (n=23) and severe (n=36) inhalation injury for the study population (n=59)

	Mild Inhalation injury (0-4 total days ventilated)					**Severe Inhalation injury** (≥ 5 total days ventilated)				
	Total samples, N (%)	**Fold change [a]**	**Mortality, N (%)**	**Fold change**	**Mortality rate (%)**	**Total samples, N (%)**	**Fold change**	**Mortality, N (%)**	**Fold change**	**Mortality rate (%)**
	23 (39)		1 (7.7)		4.3	36 (61)		14 (93.3)		38.8
BICU LOS [b]										
0-9	20 (87)	6.7	0 (0)	N/A	0	10 (27.8)	2.6	3 (21.4)	3.7	30
≥ 10	3 (13)		1 (100)		33.3	26 (72.2)		11 (78.6)		42.3
Ventilation prior to admission										
Yes	20 (87)	6.7	1 (100)	N/A	5	31 (86.1)	6.2	13 (92.9)	13	41.9
No	3 (13)		0 (0)		0	5 (13.9)		1 (7.1)		20
Arterial blood gas analysis										
pH										
< 7.35 (acidosis)	8 (34.8)		0 (0)		0	16 (44.4)		9 (64.3)		56.3
7.35-7.45 (normal)	12 (52.2)	1.5 – 4	1 (100)	N/A	8.3	16 (44.4)	4	3 (21.4)	3 – 4.5	18.8
> 7.45 (alkalosis)	3 (13)		0 (0)		0	4 (11.1)		2 (14.3)		50
PaO_2 (kPa) [c]										
< 10.5 (hypoxia)	3 (13)		0 (0)		0	6 (16.7)		3 (21.4)		50
10.5-13.5 (normal)	7 (30.4)	1.9 – 4.3	0 (0)	N/A	0	2 (5.6)	4.7 - 14	1 (7.1)	3.3 – 10	50
> 13.5 (hyperoxia)	13 (56.5)		1 (100)		7.7	28 (77.8)		10 (71.4)		35.7
PCO_2 (kPa) [d]										
< 4.7	6 (26.1)		1 (100)		16.7	7 (19.4)		3 (23.1)		42.9
4.7-6.0 (normal)	11 (47.8)	1.8	0 (0)	N/A	0	18 (50)	1.6 – 2.6	7 (53.8)	2.3	39.9
> 6.0	6 (26.1)		0 (0)		0	11 (30.6)		3 (23.1)		27.3
SATS (%)										
< 95	3 (13)	6.7	0 (0)	N/A	0	4 (11.1)	8	2 (14.3)	6	50
95-100 (normal)	20 (87)		1 (100)		5	32 (88.9)		12 (85.7)		37.5
Lactate (mmol/L)										
< 2.0 (normal)	17 (73.9)	2.8	1 (100)	N/A	5.9	17 (47.2)	1.1	5 (35.7)	1.8	29.4
≥ 2.0 (excess)	6 (26.1)		0 (0)		0	19 (52.8)		9 (64.3)		47.4
Base excess (mmol/L)										
< -4	5 (21.7)		1 (100)		20	6 (16.7)		3 (21.4)		50
-4 to +2 (normal)	15 (65.2)	3 – 5	0 (0)	N/A	0	26 (72.2)	4.3 – 6.5	9 (64.3)	3 – 4.5	34.6
> 2	3 (13)		0 (0)		0	4 (11.1)		2 (14.3)		50

[a] Fold change between more than two groups are affiliated with the highest frequency within the variable
[b] BICU LOS: burns intensive care unit length of stay
[c] PaO_2: partial pressure of arterial oxygen
[d] PCO_2: partial pressure of arterial carbon dioxide

APPENDIX C: Exemplar samples and differentially expressed miRNAs

Table 16: Demographical and pre-QC results of exemplar samples for RNA sequencing library selection

N#	Exemplar criteria information											Illumina Pre-QC results		
	Phenotypical characteristics				*Total days ventilation*	*Etiology*	*Mortality*	*Quantity [µg/µl]*	*A260/A280 Ratio (abs)*	*RIN value*	*miRNA [%]*	*Quantity [ng/µl]*	*RIN Value*	*Result*
	Singed nasal hairs	*Soot in/ around mouth*	*Facial burns*	*Hoarse*										
1	Yes	No	Yes	No	3	Fire	No	6.3	2.0	9.1	17.0	UD	7.4	Reject
2	Yes	Yes	Yes	No	4	Fire	No	15.8	2.1	9.0	12.0	0.4	8.9	Reject
3	No	Yes	Yes	No	0	Fire	No	1.4	1.8	7.7	9.0	2.7	7.4	Accept
4	Yes	Yes	Yes	Yes	2	Other	No	2.9	2.0	8.9	15.0	3.1	8.7	Accept
5	Yes	Yes	No	Yes	2	Fire	No	2.9	2.1	8.3	13.0	1.2	7.3	Accept
6	No	No	Yes	No	4	Fire	Yes	5.4	2.1	8.8	18.0	1.1	7.8	Accept
7	Yes	Yes	No	Yes	4	Fire	No	7.0	2.3	8.7	16.0	1.2	7.8	Accept
8	Yes	Yes	Yes	Yes	0	Fire	No	7.7	2.1	9.3	11.0	1.5	8.9	Accept
9	Yes	Yes	Yes	No	3	Fire	No	4.4	2.1	9.1	10.0	2.2	8.9	Accept
10	Yes	Yes	Yes	Yes	4	Fire	No	8.6	2.1	8.6	15.0	4.7	8.2	Accept
11	Yes	Yes	Yes	No	4	Fire	No	6.5	2.1	8.7	49.0	0.1	8.6	Reject
12	Yes	No	Yes	No	3	Fire	No	7.9	2.0	8.7	15.0	1.4	8.3	Accept
13	Yes	Yes	Yes	Yes	5	Fire	No	4.8	2.0	8.7	14.0	3.3	7.7	Accept
14	Yes	Yes	Yes	No	5	Fire	No	5.0	2.0	9.0	15.0	0.1	9.9	Reject
15	Yes	Yes	Yes	No	11	Fire	Yes	3.0	2.1	8.2	11.0	3.6	8.0	Accept
16	No	Yes	Yes	No	17	Fire	Yes	11.8	2.0	8.3	13.0	0.1	8.2	Reject
17	Yes	Yes	Yes	No	6	Other	No	3.6	2.1	8.5	10.0	1.9	8.9	Accept
18	Yes	Yes	Yes	Yes	5	Other	No	14.1	2.1	8.4	16.0	4.4	7.2	Accept
19	No	No	Yes	No	10	Other	No	4.4	2.1	8.8	15.0	UD	UD	Reject
20	Yes	Yes	Yes	No	16	Fire	No	2.7	2.2	9.3	12.0	0.1	8.8	Reject
21	Yes	No	Yes	No	6	Other	No	4.0	2.2	9.3	13.0	3.0	8.6	Accept
22	Yes	Yes	Yes	Yes	12	Fire	Yes	10.9	2.1	9.2	14.0	4.3	9.3	Accept
23	Yes	Yes	Yes	No	10	Fire	Yes	17.9	2.1	9.5	13.0	0.1	9.5	Reject
24	Yes	No	Yes	No	14	Other	No	4.0	2.2	8.8	10.0	4.3	8.7	Accept
25	Yes	No	Yes	No	14	Fire	Yes	5.2	2.1	8.3	9.0	4.2	8.1	Accept
26	Yes	Yes	Yes	Yes	13	Fire	No	5.6	2.1	8.7	14.0	3.3	8.2	Accept
27	Yes	Yes	Yes	Yes	5	Fire	No	3.3	2.1	8.7	17.0	9.7	8.8	Accept
28	Yes	Yes	Yes	No	12	Fire	No	7.6	2.1	8.2	47.0	2.1	8.5	Accept
29	No	No	No	No	5	Fire	Yes	11.3	2.1	9.0	11.0	3.9	9.1	Accept
30	Yes	Yes	Yes	No	16	Fire	Yes	5.2	2.1	7.6	18.0	3.1	8.1	Accept

QC – quality control, RIN – RNA integrity number, UD – Undetected.

Table 17: Statistics of reads following alignment to the human genome and miRbase data

Sample names	Raw reads	Trimmed reads	Mapped reads	% Mapped	Mature miRNA reads	Mature miRNA	Mature miRNA SA
S1_3	962310	480883	458016	96.89	443789	479	375
S2_4	590404	350757	338053	98.10	331629	439	359
S3_5	316816	253433	242776	98.30	238651	377	307
S4_6	553016	353634	338108	97.07	328193	487	378
S5_7	915426	535981	510403	96.34	491745	512	408
S6_8	10567596	8217259	7880108	98.10	7730076	846	680
S7_9	973547	768562	738759	98.32	726374	524	406
S8_10	3916180	3053362	2933287	97.60	2862832	780	609
S9_12	546676	474833	448114	96.79	433733	561	434
S10_13	1290895	1122844	1071739	96.53	1034561	676	526
S11_15	1219613	1004551	959956	97.37	934722	631	524
S12_17	1793253	909176	872539	96.85	845053	607	454
S13_18	4473654	2458111	2329588	97.10	2261918	773	603
S14_21	3553460	2528051	2401607	96.09	2307796	717	569
S15_22	12163313	5436801	5165076	96.70	4994417	802	656
S16_24	12693915	9422043	9117373	98.33	8965343	948	758
S17_25	1537391	1331643	1261322	97.18	1225691	642	514
S18_26	7730183	6800028	6560255	97.89	6421912	868	704
S19_27	6185718	4966668	4779284	97.99	4683064	863	700
S20_28	2702669	2269878	2176184	97.83	2128893	787	627
S21_29	22138349	19399306	18358415	96.97	17802525	961	800
S22_30	2298326	1957033	1866821	97.22	1814961	760	607
S1_3	962310	480883	458016	96.89	443789	479	375
S2_4	590404	350757	338053	98.10	331629	439	359
S3_5	316816	253433	242776	98.30	238651	377	307
S4_6	553016	353634	338108	97.07	328193	487	378
S5_7	915426	535981	510403	96.34	491745	512	408
S6_8	10567596	8217259	7880108	98.10	7730076	846	680

SA – sequence alignment.

Table 18: Sixty differentially expressed miRNAs in mild and severe inhalation injury using EdgeR

miRNA	Mean (CPM)		FC	P-value
	Mild	*Severe*	*(Log2)*	*(Adj.)*
hsa-miR-504-5p	8.18	2.51	-1.70	0.02
hsa-miR-1249-3p	12.89	4.94	-1.40	0.02
hsa-miR-let-7b-3p	43.04	16.58	-1.37	0.01
hsa-miR-485-3p	22.78	9.06	-1.33	0.01
hsa-miR-6511b-3p	12.86	5.38	-1.30	0.00
hsa-miR-6511b-3p	12.86	5.38	-1.29	0.00
hsa-miR-4772-3p	72.20	32.81	-1.16	0.03
hsa-miR-let-7d-3p	914.14	412.97	-1.15	0.01
hsa-miR-4742-3p	67.42	30.34	-1.12	0.00
hsa-miR-543	18.73	8.98	-1.04	0.04
hsa-miR-4685-3p	78.85	40.54	-1.00	0.01
hsa-miR-328-3p	179.32	95.69	-0.91	0.01
hsa-miR-93-3p	156.57	82.59	-0.90	0.00
hsa-miR-486-5p	299133.76	162365.24	-0.88	0.01
hsa-miR-486-5p	298204.86	161996.61	-0.88	0.01
hsa-miR-339-5p	114.31	62.50	-0.87	0.00
hsa-miR-423-5p	6487.52	3556.10	-0.87	0.04
hsa-miR-576-5p	384.17	210.510	-0.86	0.01
hsa-miR-18b-3p	22.53	12.91	-0.85	0.04
hsa-miR-484	4323.75	2433.6	-0.83	0.01
hsa-miR-3605-3p	141.85	82.44	-0.78	0.04
hsa-miR-505-3p	210.68	124.56	-0.76	0.03
hsa-miR-7706	68.56	41.39	-0.72	0.04
hsa-miR-361-5p	287.98	174.14	-0.72	0.04
hsa-miR-92b-3p	1765.66	1072.21	-0.72	0.04
hsa-miR-532-3p	108.84	67.68	-0.70	0.04
hsa-miR-197-3p	165.83	104.14	-0.68	0.03
hsa-miR-30c-5p	4986.29	3141.94	-0.67	0.04
hsa-miR-30c-5p	4984.86	3143.42	-0.66	0.04
hsa-miR-92a-3p	235520.37	156313.14	-0.59	0.04
hsa-miR-103a-3p	1755.94	2912.35	0.73	0.04
hsa-miR-103a-3p	1753.25	2908.03	0.73	0.04
hsa-miR-24-3p	127.03	219.23	0.80	0.03
hsa-miR-24-3p	129.84	225.13	0.81	0.03
hsa-miR-340-5p	30.97	57.63	0.88	<0.05
hsa-miR-27a-3p	92.41	170.82	0.88	0.04
hsa-miR-194-5p	63.85	116.97	0.88	0.01
hsa-miR-27b-3p	181.59	351.47	0.95	0.01
hsa-miR-660-5p	29.21	58.32	0.98	0.04
hsa-miR-194-5p	124.94	250.00	1.01	0.00
hsa-miR-454-3p	97.89	200.37	1.03	0.03
hsa-miR-148a-3p	547.93	1192.21	1.12	0.01
hsa-miR-16-5p	5320.77	11592.7	1.12	0.01
hsa-miR-16-5p	5327.95	11624.32	1.13	0.01
hsa-miR-101-3p	141.09	361.19	1.35	0.02
hsa-miR-101-3p	127.76	329.42	1.36	0.02
hsa-miR-126-3p	221.69	590.22	1.41	0.01
hsa-miR-7-5p	64.25	172.55	1.42	0.00
hsa-miR-7-5p	64.18	172.78	1.43	0.00
hsa-miR-30e-5p	220.00	591.02	1.43	0.00
hsa-miR-7-5p	64.21	172.99	1.43	0.00
hsa-miR-144-5p	305.61	853.37	1.48	0.03
hsa-miR-21-5p	778.9	2247.09	1.53	0.00
hsa-miR-374a-5p	44.23	138.80	1.63	0.02
hsa-miR-15a-5p	57.15	183.83	1.67	0.01
hsa-miR-30a-5p	43.37	145.89	1.74	0.02
hsa-miR-10b-5p	13.85	47.08	1.76	0.04
hsa-miR-148b-5p	4.14	13.53	1.78	0.01
hsa-miR-200b-3p	1.31	5.25	1.95	0.04
hsa-miR-143-3p	81.44	317.95	1.96	0.00

CPM – counts per million, FC – fold change, Adj. – adjusted.

Table 19: Sixty-seven differentially expressed miRNAs in mild and severe inhalation injury using DESeq2

miRNA	Mean (CPM)		FC	P-value
	Mild	*Severe*	*(Log2)*	*(Adj.)*
hsa-miR-504-5p	8.09	2.60	-1.68	0.03
hsa-let-7b-3p	41.91	16.34	-1.36	0.01
hsa-miR-1249-3p	13.15	5.02	-1.35	0.01
hsa-miR-1908-5p	9.00	4.20	-1.30	0.03
hsa-miR-485-3p	22.47	9.23	-1.30	0.02
hsa-miR-6511b-3p	12.10	5.47	-1.24	0.01
hsa-miR-6511b-3p	12.10	5.47	-1.24	0.01
hsa-miR-4772-3p	69.48	32.39	-1.15	0.03
hsa-let-7d-3p	881.64	411.6	-1.10	0.02
hsa-miR-4742-3p	66.20	30.15	-1.08	0.00
hsa-miR-4685-3p	75.43	41.33	-0.95	0.01
hsa-miR-328-3p	173.11	96.21	-0.86	0.02
hsa-miR-93-3p	152.22	82.64	-0.84	0.00
hsa-miR-339-5p	110.35	62.25	-0.84	0.00
hsa-miR-576-5p	371.84	208.67	-0.83	0.01
hsa-miR-423-5p	6273.98	3538.03	-0.83	<0.05
hsa-miR-486-5p	288456.08	163041.91	-0.82	0.01
hsa-miR-486-5p	287559.67	162672.27	-0.82	0.01
hsa-miR-18b-3p	21.39	13.10	-0.8	0.04
hsa-miR-484	4196.68	2427.56	-0.79	0.00
hsa-miR-505-3p	203.30	124.38	-0.71	0.03
hsa-miR-361-5p	277.99	172.44	-0.68	0.04
hsa-miR-7706	66.57	41.35	-0.68	0.03
hsa-miR-532-3p	105.49	68.26	-0.65	0.04
hsa-miR-30c-5p	4825.20	3105.10	-0.63	0.02
hsa-miR-30c-5p	4823.72	3106.57	-0.63	0.02
hsa-miR-197-3p	159.15	104.57	-0.62	0.02
hsa-miR-92a-3p	227678.99	156811.66	-0.54	0.03
hsa-miR-425-5p	6735.38	9247.63	0.46	0.03
hsa-miR-652-3p	1185.01	1663.18	0.49	0.02
hsa-miR-22-3p	1273.59	2044.06	0.68	0.03
hsa-miR-103a-3p	1688.41	2924.74	0.79	0.01
hsa-miR-103a-3p	1690.89	2929.09	0.79	0.01
hsa-miR-503-5p	160.47	281.87	0.81	0.03
hsa-miR-152-3p	17.18	32.55	0.83	<0.05
hsa-miR-93-5p	3278.93	5853.02	0.84	0.04
hsa-miR-17-5p	87.38	158.14	0.86	0.03
hsa-miR-24-3p	122.92	219.61	0.86	0.01
hsa-miR-24-3p	125.64	225.42	0.87	0.01
hsa-miR-340-5p	29.25	57.32	0.92	0.03
hsa-miR-194-5p	61.50	116.39	0.93	0.00
hsa-miR-27a-3p	88.54	171.84	0.94	0.02
hsa-miR-106b-5p	167.22	325.64	0.95	0.04
hsa-miR-126-5p	36.73	73.02	0.98	0.04
hsa-miR-27b-3p	173.13	352.24	1.01	0.00
hsa-miR-660-5p	27.34	58.00	1.04	0.03
hsa-miR-194-5p	120.88	248.37	1.06	0.00
hsa-miR-454-3p	92.63	201.26	1.10	0.02
hsa-miR-148a-3p	525.48	1190.15	1.18	0.00
hsa-miR-16-5p	5122.47	11630.36	1.18	0.00
hsa-miR-16-5p	5129.30	11661.94	1.18	0.01
hsa-miR-101-3p	133.10	367.73	1.45	0.01
hsa-miR-101-3p	120.48	335.59	1.46	0.01
hsa-miR-126-3p	212.36	601.53	1.50	0.01
hsa-miR-7-5p	61.34	173.78	1.50	0.00
hsa-miR-30e-5p	211.06	595.13	1.50	0.00
hsa-miR-7-5p	61.28	174.00	1.50	0.00
hsa-miR-7-5p	61.31	174.21	1.50	0.00
hsa-miR-144-5p	290.55	876.24	1.59	0.01
hsa-miR-21-5p	744.64	2255.58	1.60	0.00
hsa-miR-374a-5p	41.47	137.89	1.70	0.01
hsa-miR-15a-5p	54.45	185.66	1.74	0.00
hsa-miR-10b-5p	13.94	46.03	1.78	0.03
hsa-miR-30a-5p	42.18	146.59	1.81	0.02
hsa-miR-148b-5p	4.69	13.26	1.84	0.00
hsa-miR-200b-3p	1.40	5.02	1.93	0.02
hsa-miR-143b-3p	78.69	315.29	2.02	0.00

CPM – counts per million, FC – fold change, Adj. – adjusted.

Table 17: Fifty-seven differentially expressed miRNAs in mild and severe inhalation injury that overlap using EdgeR and DESeq2

miRNA	EdgeR				DESeq2			
	Mean (CPM)		FC	P-value	Mean (CPM)		FC	P-value
	Mild	*Severe*	(Log2)	(Adj.)	*Mild*	*Severe*	(Log2)	(Adj.)
hsa-miR-504-5p	8.18	2.51	-1.70	0.02	8.09	2.60	-1.68	0.03
hsa-miR-1249-3p	12.89	4.94	-1.40	0.02	13.15	5.02	-1.35	0.01
hsa-miR-let-7b-3p	43.04	16.58	-1.37	0.01	41.91	16.34	-1.36	0.01
hsa-miR-485-3p	22.78	9.06	-1.33	0.02	22.47	9.23	-1.30	0.02
hsa-miR-6511b-3p	12.86	5.38	-1.30	0.00	12.10	5.47	-1.24	0.01
hsa-miR-6511b-3p	12.86	5.38	-1.29	0.00	12.10	5.47	-1.24	0.01
hsa-miR-4772-3p	72.20	32.81	-1.16	0.03	69.48	32.39	-1.15	0.03
hsa-miR-let-7d-3p	914.14	412.97	-1.15	0.01	881.64	411.6	-1.10	0.02
hsa-miR-4742-3p	67.42	30.34	-1.12	0.00	66.20	30.15	-1.08	0.00
hsa-miR-4685-3p	78.85	40.54	-1.00	0.01	75.43	41.33	-0.95	0.01
hsa-miR-328-3p	179.32	95.69	-0.91	0.01	173.11	96.21	-0.86	0.02
hsa-miR-93-3p	156.57	82.59	-0.90	0.00	152.22	82.64	-0.84	0.00
hsa-miR-486-5p	299133.76	162365.24	-0.88	0.01	288456.08	163041.91	-0.82	0.01
hsa-miR-486-5p	298204.86	161996.61	-0.88	0.01	287559.67	162672.27	-0.82	0.01
hsa-miR-339-5p	114.31	62.50	-0.87	0.00	110.35	62.25	-0.84	0.00
hsa-miR-423-5p	6487.52	3556.10	-0.87	0.04	6273.98	3538.03	-0.83	0.04
hsa-miR-576-5p	384.17	210.510	-0.86	0.01	371.84	208.67	-0.83	0.01
hsa-miR-18b-3p	22.53	12.91	-0.85	0.04	21.39	13.10	-0.80	0.04
hsa-miR-484	4323.75	2433.6	-0.83	0.01	4196.68	2427.56	-0.79	0.00
hsa-miR-505-3p	210.68	124.56	-0.76	0.03	203.30	124.38	-0.71	0.02
hsa-miR-7706	68.56	41.39	-0.72	0.04	66.57	41.35	-0.68	0.03
hsa-miR-361-5p	287.98	174.14	-0.72	0.04	277.99	172.44	-0.68	0.03
hsa-miR-532-3p	108.84	67.68	-0.70	0.04	105.49	68.26	-0.65	0.04
hsa-miR-197-3p	165.83	104.14	-0.68	0.03	159.15	104.57	-0.62	0.02
hsa-miR-30c-5p	4986.29	3141.94	-0.67	0.04	4825.20	3105.10	-0.63	0.02
hsa-miR-30c-5p	4984.86	3143.42	-0.66	0.04	4823.72	3106.57	-0.63	0.02
hsa-miR-92a-3p	235520.37	156313.14	-0.59	0.04	227678.99	156811.66	-0.54	0.03
hsa-miR-103a-3p	1755.94	2912.35	0.73	0.04	1688.41	2924.74	0.79	0.01
hsa-miR-103a-3p	1753.25	2908.03	0.73	0.04	1690.89	2929.09	0.79	0.01
hsa-miR-24-3p	127.03	219.23	0.80	0.03	122.92	219.61	0.86	0.01
hsa-miR-24-3p	129.84	225.13	0.81	0.03	125.64	225.42	0.87	0.01
hsa-miR-340-5p	30.97	57.63	0.88	0.05	29.25	57.32	0.92	0.03
hsa-miR-27a-3p	92.41	170.82	0.88	0.04	88.54	171.84	0.94	0.02
hsa-miR-194-5p	63.85	116.97	0.88	0.01	61.50	116.39	0.93	0.00
hsa-miR-27b-3p	181.59	351.47	0.95	0.01	173.13	352.24	1.01	0.00
hsa-miR-660-5p	29.21	58.32	0.98	0.05	27.34	58.00	1.04	0.03
hsa-miR-194-5p	124.94	250.00	1.01	0.00	120.88	248.37	1.06	0.00
hsa-miR-454-3p	97.89	200.37	1.03	0.03	92.63	201.26	1.10	0.01
hsa-miR-148a-3p	547.93	1192.21	1.12	0.01	525.48	1190.15	1.18	0.00
hsa-miR-16-5p	5320.77	11592.7	1.12	0.01	5122.47	11630.36	1.18	0.00
hsa-miR-16-5p	5327.95	11624.32	1.13	0.01	5129.30	11661.94	1.18	0.00
hsa-miR-101-3p	141.09	361.19	1.35	0.02	133.10	367.73	1.45	0.01
hsa-miR-101-3p	127.76	329.42	1.36	0.02	120.48	335.59	1.46	0.01
hsa-miR-126-3p	221.69	590.22	1.41	0.01	212.36	601.53	1.50	0.01
hsa-miR-7-5p	64.25	172.55	1.42	0.00	61.28	174.00	1.50	0.00
hsa-miR-7-5p	64.18	172.78	1.43	0.00	61.31	174.21	1.50	0.00
hsa-miR-30e-5p	220.00	591.02	1.43	0.00	211.06	595.13	1.50	0.00
hsa-miR-7-5p	64.21	172.99	1.43	0.00	61.34	173.78	1.50	0.00
hsa-miR-144-5p	305.61	853.37	1.48	0.03	290.55	876.24	1.59	0.01
hsa-miR-21-5p	778.9	2247.09	1.53	0.00	744.64	2255.58	1.60	0.00
hsa-miR-374a-5p	44.23	138.80	1.63	0.02	41.47	137.89	1.70	0.01
hsa-miR-15a-5p	57.15	183.83	1.67	0.01	54.45	185.66	1.74	0.00
hsa-miR-30a-5p	43.37	145.89	1.74	0.02	42.18	146.59	1.81	0.02
hsa-miR-10b-5p	13.85	47.08	1.76	0.04	13.94	46.03	1.78	0.03
hsa-miR-148b-5p	4.14	13.53	1.78	0.01	4.69	13.26	1.84	0.00
hsa-miR-200b-3p	1.31	5.25	1.95	0.04	1.40	5.02	1.93	0.02
hsa-miR-143-3p	81.44	317.95	1.96	0.00	78.69	315.29	2.02	0.00

CPM – counts per million, FC – fold change, Adj. – adjusted.

APPENDIX D: Target genes, protein-protein networks and functionally enriched pathways

Table 21: Topology features and miRNA nodes for each hub gene in the up-regulated miRNA-mRNA network

ID	Degree; Betweenness	Nodes (miRNA connections)	ID	Degree; Betweenness	Nodes (miRNA connections)
NUFIP2	6; 59968.5	miR-15a-5p, miR-21-5p, miR-30a-5p, miR-374a-5p, miR-200-3p, miR-10b-5p	PLEKHA1	3; 17022.2	miR-15a-5p, miR-21-5p, miR-143-3p
NR2C2	5; 47561.8	miR-15a-5p, miR-21-5p, miR-374a-5p, miR-143-3p, miR-10b-5p	KLHL15	3; 9986.5	miR-15a-5p, miR-21-5p, miR-374a-5p
BCL2	4; 26444.5	miR-15a-5p, miR-21-5p, miR-143-3p, miR-200-3p	SELENOI	3; 12924.9	miR-15a-5p, miR-30a-5p, miR-374a-5p
VEGFA	4; 18358.2	miR-15a-5p, miR-21-5p, miR-374a-5p, miR-200-3p	SESTD1	3; 9986.5	miR-15a-5p, miR-21-5p, miR-374a-5p
CCNE2	4; 21812.1	miR-15a-5p, miR-30a-5p, miR-144-5p, miR-200-3p	MKNK2	3; 11861.6	miR-21-5p, miR-10b-5p, miR-200-3p
ZNF460	4; 31561.5	miR-15a-5p, miR-21-5p, miR-30a-5p, miR-10b-5p	IGF1R	3; 18588.0	miR-21-5p, miR-30a-5p, miR-143-3p
SEC24A	4; 20551.5	miR-15a-5p, miR-30a-5p, miR-374a-5p, miR-200-3p	MBNL1	3; 16482.1	miR-21-5p, miR-30a-5p, miR-10b-5p
RAB21	4; 20551.5	miR-15a-5p, miR-30a-5p, miR-374a-5p, miR-200-3p	SP1	3; 8617.2	miR-21-5p, miR-374a-5p, miR-200-3p
MIB1	4; 25693.2	miR-15a-5p, miR-21-5p, miR-30a-5p, miR-374a-5p	WNT5A	3; 12409.6	miR-21-5p, miR-30a-5p, miR-374a-5p
PTAR1	4; 25693.2	miR-15a-5p, miR-21-5p, miR-30a-5p, miR-374a-5p	SECISBP2L	3; 13140.8	miR-21-5p, miR-143-3p, miR-200-3p
ATP5G3	3; 256312.0	miR-15a-5p, miR-148a-5p, miR-200-3p	NFAT5	3; 12409.6	miR-21-5p, miR-30a-5p, miR-374a-5p
BMI1	3; 10528.9	miR-15a-5p, miR-21-5p, miR-200-3p	FRS2	3; 13594.5	miR-21-5p, miR-30a-5p, miR-200-3p
BRCA1	3; 13991.9	miR-15a-5p, miR-21-5p, miR-10b-5p	HIC2	3; 16920.0	miR-21-5p, miR-30a-5p, miR-148a-5p
E2F3	3; 10528.9	miR-15a-5p, miR-21-5p, miR-200-3p	TRIM2	3; 12409.6	miR-21-5p, miR-374a-5p, miR-10b-5p
JUN	3; 11988.2	miR-15a-5p, miR-30a-5p, miR-200-3p	HECTD1	3; 12409.6	miR-21-5p, miR-30a-5p, miR-374a-5p
PIK3R1	3; 17022.2	miR-15a-5p, miR-21-5p, miR-143-3p	LIN7C	3; 12409.6	miR-21-5p, miR-30a-5p, miR-374a-5p
MAP2K3	3; 16065.5	miR-15a-5p, miR-21-5p, miR-30a-5p	BTBD7	3; 12379.6	miR-21-5p, miR-30a-5p, miR-374a-5p
TP53	3; 16583.5	miR-15a-5p, miR-30a-5p, miR-10b-5p	KLHL42	3; 9055.9	miR-21-5p, miR-144-5p, miR-200-3p
TUBB2A	3; 12197.2	miR-15a-5p, miR-143-3p, miR-200-3p	KBTBD6	3; 16482.1	miR-21-5p, miR-30a-5p, miR-10b-5p
UGDH	3; 16583.5	miR-15a-5p, miR-30a-5p, miR-10b-5p	AHSA2	3; 12060.1	miR-21-5p, miR-374a-5p, miR-10b-5p
SHOC2	3; 11934.5	miR-15a-5p, miR-374a-5p, miR-200-3p	GK5	3; 12060.1	miR-21-5p, miR-374a-5p, miR-10b-5p
FZD6	3; 9986.5	miR-15a-5p, miR-21-5p, miR-374a-5p	ACSL4	3; 8605.9	miR-30a-5p, miR-374a-5p, miR-200-3p
CBX4	3; 9986.5	miR-15a-5p, miR-21-5p, miR-374a-5p	NOTCH1	3; 11503.5	miR-30a-5p, miR-200-3p, miR-10b-5p
BAG4	3; 11988.2	miR-15a-5p, miR-30a-5p, miR-200-3p	FSCN1	3; 12361.8	miR-30a-5p, miR-143-3p, miR-10b-5p
NCKAP1	3; 19192.9	miR-15a-5p, miR-30a-5p, miR-143-3p	STX16	3; 8605.9	miR-30a-5p, miR-374a-5p, miR-200-3p
DICER1	3; 9986.5	miR-15a-5p, miR-21-5p, miR-374a-5p	ZNF264	3; 17421.8	miR-30a-5p, miR-148a-5p, miR-143-3p
CADM1	3; 13991.9	miR-15a-5p, miR-21-5p, miR-10b-5p	ROCK2	3; 7912.1	miR-30a-5p, miR-144-5p, miR-200-3p
TMEM245	3; 9986.5	miR-15a-5p, miR-21-5p, miR-374a-5p	MBNL2	3; 13181.1	miR-30a-5p, miR-374a-5p, miR-10b-5p
AP3M1	3; 13991.9	miR-15a-5p, miR-21-5p, miR-10b-5p	PABPC1	3; 11503.5	miR-30a-5p, miR-200-3p, miR-10b-5p
AGO2	3; 16065.5	miR-15a-5p, miR-21-5p, miR-30a-5p	MIER3	3; 15756.9	miR-30a-5p, miR-374a-5p, miR-143-3p
PDCD4	3; 13991.9	miR-15a-5p, miR-21-5p, miR-10b-5p	XIAP	3; 9534.3	miR-143-3p, miR-200-3p, miR-10b-5p
PNPO	3; 19192.9	miR-15a-5p, miR-30a-5p, miR-143-3p	BCL2L11	3; 10322.1	miR-148a-5p, miR-200-3p, miR-10b-5p
ETNK1	3; 9986.5	miR-15a-5p, miR-21-5p, miR-374a-5p	SFT2D2	3; 14313.0	miR-374a-5p, miR-143-3p, miR-10b-5p
TAOK1	3; 14514.0	miR-15a-5p, miR-30a-5p, miR-145-5p	MAPK7	3; 10505.6	miR-374a-5p, miR-143-3p, miR-200-3p

Table 22: Topology features and miRNA nodes for each hub gene in the combined (up- and down-regulated) miRNA-mRNA network

ID	Degree; Betweenness	Nodes (miRNA connections)	ID	Degree; Betweenness	Nodes (miRNA connections)
NR2C2	6; 61054.5	miR-15a-5p, miR-143-3p, miR-374a-5p, miR-21-5p, miR-10b-5p, miR-504-5p	TAOK1	3; 14654.6	miR-15a-5p, miR-144-3p, miR-30a-5p
NUFIP2	6; 59155.3	miR-15a-5p, miR-374a-5p, miR-21-5p, miR-30a-5p, miR-10b-5p, miR-200b-3p	PLEKHA1	3; 16916.6	miR-15a-5p, miR-143-3p, miR-21-5p,
VEGFA	5; 30458.3	miR-15a-5p, miR-374a-5p, miR-21-5p, miR-504-5p, miR-200b-3p	KLHL15	3; 9960.0	miR-15a-5p,miR-374a-5p,miR-21-5p
BCL2	4; 26251.6	miR-15a-5p, miR-143-3p, miR-21-5p, miR-200b-3p	SELENOI	3; 12886.5	miR-15a-5p,miR-374a-5p,miR-30a-5p
TP53	4; 26405.7	miR-15a-5p, miR-30a-5p, miR-504-5p, miR-10b-5p	SESTD1	3; 9960.0	miR-15a-5p,miR-374a-5p,miR-21-5p
CCNE2	4; 21962.6	miR-15a-5p, miR-21-5p, miR-30a-5p, miR-10b-5p	RAB15	3; 9322.2	miR-15a-5p, miR-504-5p, miR-10b-5p
ZNF460	4; 31157.3	miR-15a-5p, miR-21-5p, miR-30a-5p, miR-10b-5p	MKNK2	3; 11603.2	miR-21-5p,miR-10b-5p,miR-200b-3p
SEC24A	4; 20453.3	miR-15a-5p, miR-374a-5p, miR-30a-5p, miR-200b-3p	IGF1R	3; 18498.3	miR-143-3p, miR-21-5p,miR-30a-5p
RAB21	4; 20453.3	miR-15a-5p, miR-374a-5p, miR-30a-5p, miR-200b-3p	MBNL1	3; 16241.0	miR-21-5p,miR-30a-5p,miR-10b-5p
AGO2	4; 30283.2	miR-15a-5p, miR-21-5p, miR-30a-5p, miR-504-5p	SP1	3; 8558.8	miR-374a-5p,miR-21-5p,miR-200b-3p
MIB1	4; 256312.0	miR-15a-5p, miR-374a-5p, miR-21-5p, miR-30a-5p	WNT5A	3; 12379.6	miR-374a-5p,miR-21-5p,miR-30a-5p
PTAR1	4; 25632.0	miR-15a-5p, miR-374a-5p, miR-21-5p, miR-30a-5p	SECISBP2L	3; 13031.1	miR-143-3p, miR-21-5p,miR-200b-3p
TRIM2	4; 19221.5	miR-374a-5p, miR-21-5p, miR-504-5p, miR-10b-5p	NFAT5	3; 12379.6	miR-374a-5p,miR-21-5p,miR-30a-5p
BCL2L11	4; 14888.0	miR-504-5p, miR-10b-5p, miR-200b-3p, miR-148b-5p	FRS2	3; 13530.5	miR-21-5p,miR-30a-5p,miR-200b-3p
ACVR2A	3; 9322.2	miR-15a-5p, miR-504-5p, miR-10b-5p	HIC2	3; 16894.4	miR-21-5p,miR-30a-5p,miR-148b-5p
ATP5G3	3; 10998.2	miR-200b-3p,miR-148b-5p, miR-15a-5p	HECTD1	3; 12379.6	miR-374a-5p,miR-21-5p,miR-30a-5p
BMI1	3; 10472.3	miR-15a-5p, miR-21-5p, miR-200b-3p	LIN7C	3; 12379.6	miR-374a-5p,miR-21-5p,miR-30a-5p
BRCA1	3; 13726.0	miR-15a-5p, miR-21-5p, miR-10b-5p	BTBD7	3; 12379.6	miR-374a-5p,miR-21-5p,miR-30a-5p
CDK6	3; 12364.3	miR-15a-5p, miR-21-5p, miR-504-5p	KLHL42	3; 9134.3	miR-21-5p,miR-144-3p, miR-200b-3p
E2F3	3; 10472.3	miR-15a-5p, miR-21-5p, miR-200b-3p	KBTBD6	3; 16241.0	miR-21-5p,miR-30a-5p,miR-10b-5p
JUN	3; 11934.5	miR-15a-5p, miR-30a-5p, miR-200b-3p	AHSA2	3; 11764.5	miR-374a-5p,miR-21-5p,miR-10b-5p
PIK3R1	3; 16916.6	miR-15a-5p, miR-143-3p, miR-21-5p,	GK5	3; 11764.5	miR-374a-5p,miR-21-5p,miR-10b-5p
MAP2K3	3; 16038.0	miR-15a-5p, miR-21-5p, miR-30a-5p	ACSL4	3; 8553.0	miR-374a-5p,miR-30a-5p,miR-200b-3p
TUBB2A	3; 12083.3	miR-15a-5p, miR-143-3p, miR-200b-3p	NOTCH1	3; 11254.7	miR-30a-5p,miR-10b-5p,miR-200b-3p
UGDH	3; 16309.7	miR-15a-5p, miR-30a-5p, miR-10b-5p	FSCN1	3; 12265.8	miR-30a-5p,miR-200b-3p,miR-143-3p
SHOC2	3; 7532.6	miR-15a-5p,miR-374a-5p,miR-200b-3p	STX16	3; 8553.0	miR-374a-5p,miR-30a-5p,miR-200b-3p
FZD6	3; 9960.0	miR-15a-5p,miR-374a-5p,miR-21-5p	ZNF264	3; 17319.5	miR-143-3p,miR-30a-5p,miR-148b-5p
CBX4	3; 9960.0	miR-15a-5p,miR-374a-5p,miR-21-5p	ROCK2	3; 8000.2	miR-144-3p, miR-30a-5p,miR-200b-3p
BAG4	3; 11934.5	miR-15a-5p,miR-30a-5p,miR-200b-3p	MBNL2	3; 12880.2	miR-374a-5p,miR-30a-5p,miR-10b-5p
NCKAP1	3; 19083.5	miR-15a-5p, miR-143-3p, miR-30a-5p,	PABPC1	3; 11254.7	miR-30a-5p,miR-10b-5p,miR-200b-3p
DICER1	3; 9960.0	miR-15a-5p,miR-374a-5p,miR-21-5p	TMED5	3; 11595.1	miR-30a-5p,miR-504-5p, miR-10b-5p
CADM1	3; 13726.0	miR-15a-5p, miR-21-5p, miR-10b-5p	MIER3	3; 15625.3	miR-143-3p,miR-374a-5p,miR-30a-5p
TMEM245	3; 9960.0	miR-15a-5p,miR-374a-5p,miR-21-5p	XIAP	3; 9247.8	miR-143-3p,miR-200b-3p,miR-143-3p
SERBP1	3; 9227.8	miR-15a-5p,miR-374a-5p,miR-504-5p	RORA	3; 6839.8	miR-374a-5p,miR-504-5p,miR-10b-5p
AP3M1	3; 13726.0	miR-15a-5p, miR-21-5p, miR-10b-5p	CRLF3	3; 4776.0	miR-504-5p,miR-10b-5p,miR-200b-3p
PDCD4	3; 13726.0	miR-15a-5p, miR-21-5p, miR-10b-5p	SFT2D2	3; 13919.6	miR-143-3p,miR-374a-5p,miR-10b-5p,
PNPO	3; 19083.5	miR-15a-5p, miR-143-3p, miR-30a-5p,	ANKRD33B	3; 4776.0	miR-504-5p,miR-10b-5p,miR-200b-3p
ETNK1	3; 9960.0	miR-15a-5p,miR-374a-5p,miR-21-5p	MAPK7	3; 10370.0	miR-143-3p,miR-374a-5p,miR-200b-3p

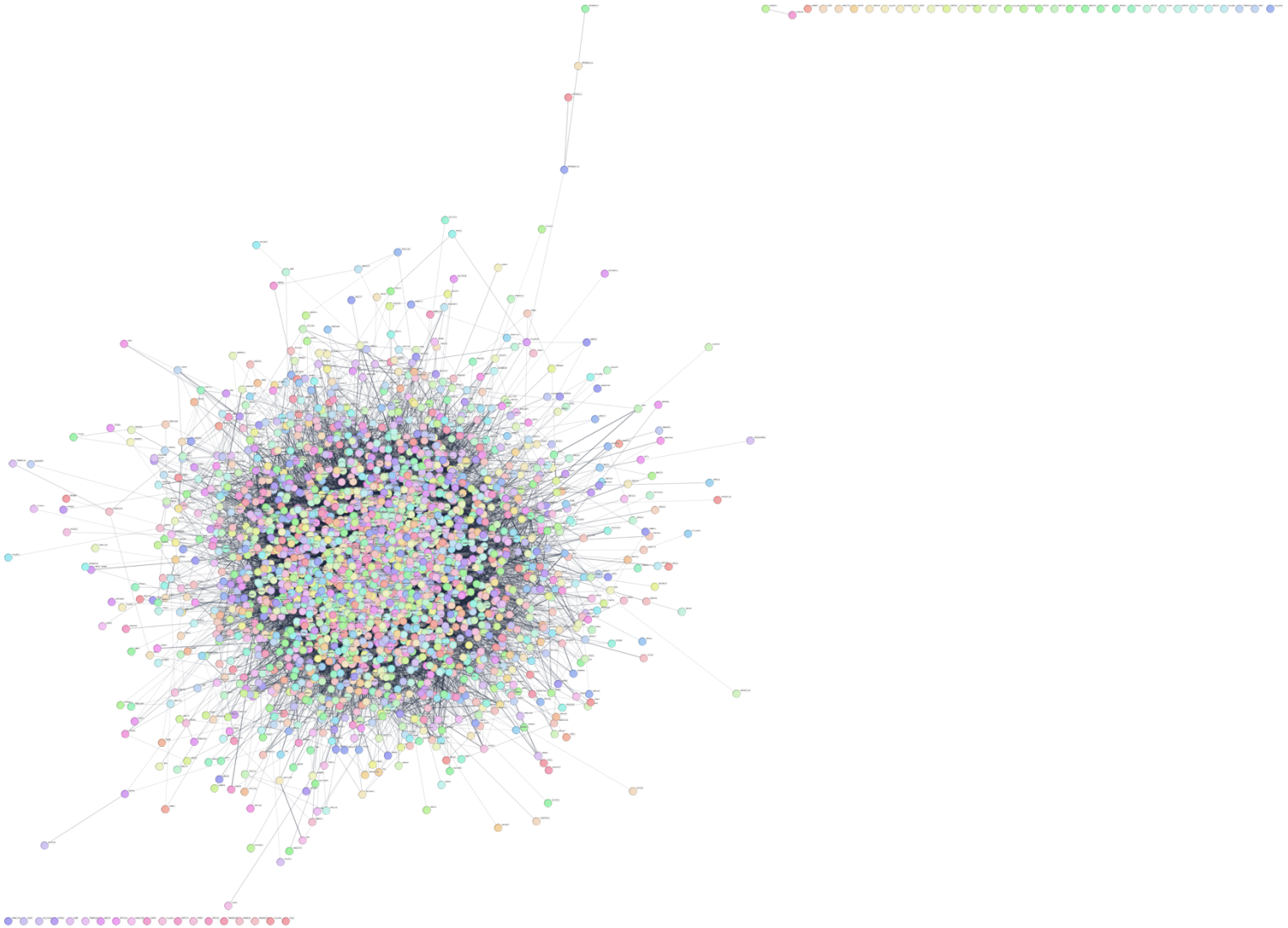


Figure 13: Protein-protein interaction network for the target genes (nodes=2637, edges=46432) of the up-regulated miRNAs (n=9).

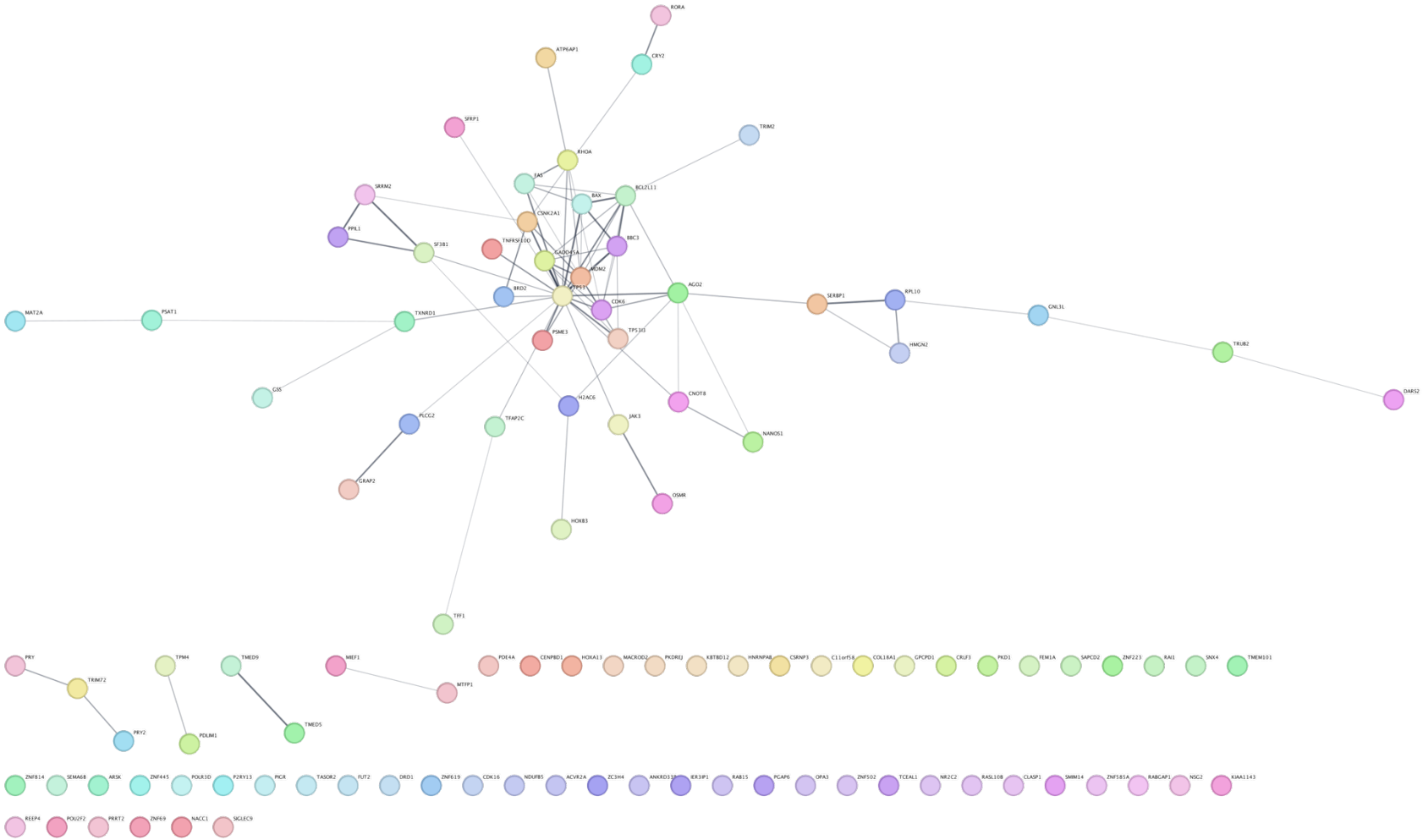


Figure 14: Protein-protein interaction network for the target genes (nodes=107, edges=80) of the down-regulated miRNAs (n=1).

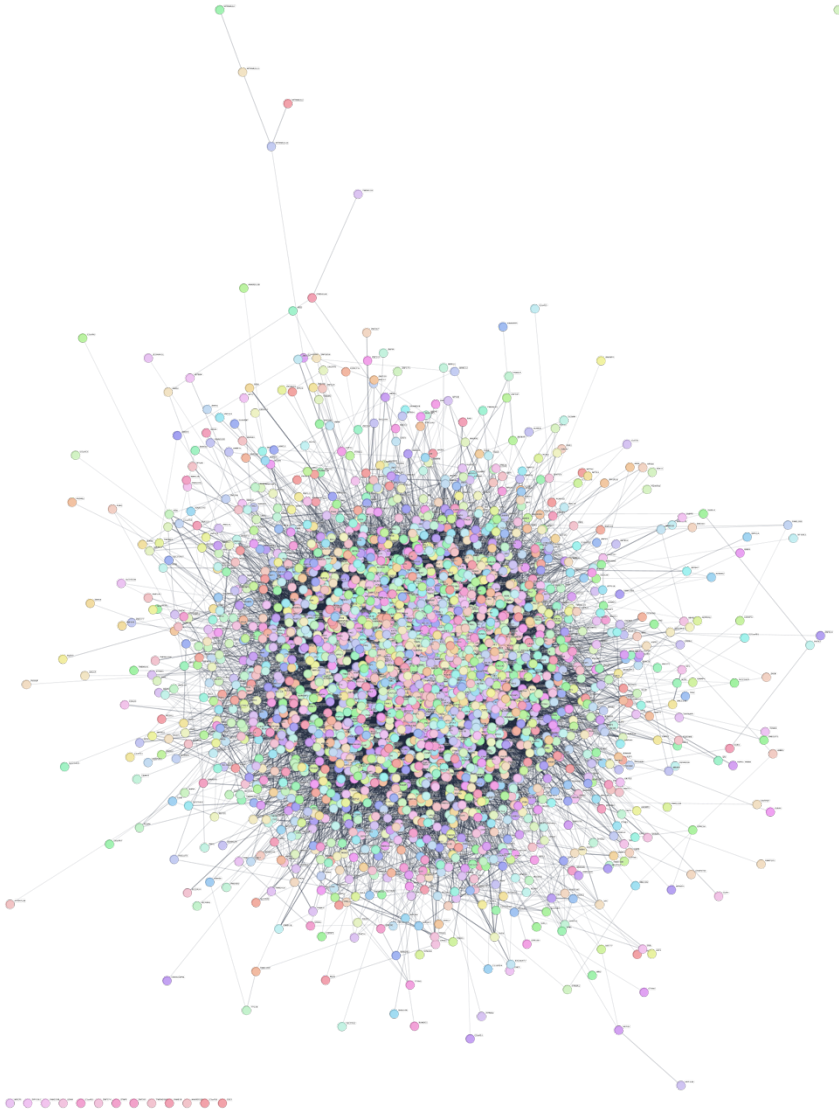


Figure 15: Protein-protein interaction network for the target genes (nodes=2692, edges= 47670) of the up and down-regulated miRNAs (n=10).