Reticulocyte Maturation Index: A Prediction Tool for Recovery in Post Bone Marrow and Peripheral Blood Stem Cell Transplant Patients

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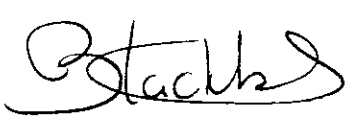
I declare that this thesis is my own work. It is being submitted for the Master's Degree in Technology (Medical Technology) to the Cape Technikon, Cape Town.

It has not previously been submitted for any diplomas, degrees or examination at any other institution.

This work was carried out in the Department of Haematology, UCT Medical School and Groote Schuur Hospital.

The opinions and conclusions drawn are not necessarily those of the Cape Technikon.



30/05/02

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Date

In Loving memory of my Mother

Sheila Josephine Blackbeard

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ABSTRACT

Erythropoietic response is the first indication of bone marrow recovery following bone marrow or peripheral blood stem cell transplantation. Manual reticulocyte counting has not only proven to be outdated but an extremely crude method of analysis, particularly if accurate and reliable means of assessing erythroid response is required to assess bone marrow recovery. Automated methods allow for the quantification of maturation within each reticulocyte, by measuring the amount of **RNA** present. The method of choice for our reticulocyte analysis was the Reticulocyte Maturation Index (**RMI**). The **RMI** was obtained by dividing the number of immature reticulocytes counted by the total number of reticulocytes counted producing a reportable value of International Units (**IU**). A normal Reticulocyte Maturation Index is 0.20 to 0.50 ıU.

The aim of the study was multifold. We wanted to prove that the Reticulocyte Maturation Index (**RMI**) is indeed the fastest means to assess bone marrow recovery in various types of transplants, including Bone Marrow Transplant (**BMT**) and Peripheral Blood Stem Cell Transplant (**PBSCT**). We also wanted to draw comparisons between allogeneic and autologous transplants, as well as further assessing different disease types. This was done by measuring the Reticulocyte Maturation Index (**RMI**), Absolute Neutrophil Count (**ANC**) and the Platelet Count (**PLT**) within the various groups. We further wanted to assess the effect of preconditioning treatment, Mononuclear Counts (**MNC**) and Colony Forming Unit – Granulocyte and Monocyte Counts (**CFU-GM**) on the early **RMI** response. These comparisons resulted in a need to establish a working range to determine patients response therein, and final outcome of the transplants. Finally we wanted to establish whether the "day 14" marrow biopsy is necessary, particularly if the three peripheral blood parameters, **RMI**, **ANC** and **PLT** were used as routine procedure following transplantation.

The Reticulocyte Maturation Index (**RMI**) was measured on the Coulter **EPICS Profile II** flow cytometer; the **ANC** and **PLT** were measured on the Technicon H^{2*} Haematology

System. All other results such as the Mononuclear Counts (**MNC**), Colony Forming Unit – Granulocyte and Monocyte counts (**CFU-GM**), "day 14" and "day 28" bone marrow biopsies were retrieved from laboratory records.

Forty nine transplant patients were evaluated for **RMI** over a period of six months, at the Department of Haematology, Groote Schuur Hospital, Cape Town. Four patients failed to engraft; and were not used in the calculations; but were evaluated as an aspect of the study in the final analysis. Forty five patients were analysed to establish the values used in the study, these patients were divided into eleven groups. Forty five total transplants, consisting of thirty two allogeneic transplants and thirteen were autologous. Ten Bone Marrow Transplant (**BMT**) patients were compared to thirty five Peripheral Blood Stem Cell Transplant (**PBSCT**) patients. The Peripheral Blood Stem Cell Transplant (**PBSCT**) group was subdivided into twenty two allogenic Peripheral Blood Stem Cell Transplants (allo**PBSCT**) and thirteen autologous Peripheral Blood Stem Cell Transplants (auto**PBSCT**). In addition, there were sufficient numbers to further evaluate eight Acute Myeloid Leukaemia allogenic Peripheral Blood Stem Cell Transplants (**AML** allo**PBSCT**), seven Chronic Myelocytic Leukaemia allogenic Peripheral Blood Stem Cell Transplants (**CML** allo**PBSCT**) and nine Acute Myeloid Leukaemia autologous Peripheral blood Stem Cell Transplants (**AML** auto**PBSCT**).

Of the original forty nine patients analysed, twenty two achieved a "12 month post transplant trilineage response".

Five of the eleven groups examined produced a mean Reticulocyte Maturation Index (**RMI**) Response Time of 0.20 IU within eight days; ranging from eight to eleven days. Four of the eleven groups produced a mean Absolute Neutrophil Count (**ANC**) Response Time of $0.50 \times 10^9/l$ within sixteen days, ranging from thirteen to twenty days. The Platelet Count (**PLT**) mean Response Time of $50 \times 10^9/l$ varied from twelve to twenty one days.

Of the forty five patients analysed 95% generated a 0.20 IU **RMI** response within sixteen days, the groups varied between fourteen and seventeen days (this response was termed a Point of Response). All the patients that engrafted did so within seventeen days. The four patients that failed to engraft never achieved a seventeen day Point of Response. The 0.50 $x10^9$/l Absolute Neutrophil Count (**ANC**) 95% Point of Response ranged from twenty one to thirty days within the various groups. The Peripheral Blood Stem Cell Transplant (**PBSCT**), autologous transplant group and the autologous Peripheral Blood Stem Cell Transplant (auto**PBSCT**) being the earliest groups to respond (twenty one days). The 0.50 $x10^9$/l Platelet Count (**PLT**) 95% Point of Response, varied between twenty three and thirty six days for the various groups. The Acute Myeloid Leukaemia autologous Peripheral Blood Stem Cell Transplant (**AML** auto**PBSCT**) group had the earliest response (twenty three days) and the Chronic Myeloid Leukaemia allogenic Peripheral Blood Stem Cell Transplant (**CML** allo**PBSCT**) had the slowest (thirty six days).

A combination of Cyclophosphamide and Total Body Irradiation and Total Nodal Irradiation (**Cyclo/TBI/TNI**) was the preconditioning used in forty three percent of the patients prior to transplant. Seventy percent of them generated a response prior to the mean Response Time in all three peripheral blood parameters (**RMI**, **ANC** and **PLT**). Only forty percent of them were still alive after a year, this was termed a "12 month post transplant trilineage response". Although five percent more allogeneic transplants generated earlier responses than autologous transplants, the autologous transplants achieved a one hundred percent "12 month post transplant trilineage response". Eleven percent more **PBSCT** generated earlier responses than the **BMT**, with fifty percent of the patients achieving a "12 month post transplant trilineage response" compared to the thirty percent **BMT**. Although twenty percent more allo**PBSCT** generated earlier responses than auto**PBSCT**, the auto**PBSCT** produced increased positive medium successes. One hundred percent achieved a "12 month post transplant trilineage response". The smaller groups although (**AML** allo**PBSCT**, the **CML** allo**PBSCT** and the **AML** auto**PBSCT**), generating early responses in sixty percent of their patients. The best medium term

outcome was the **AML** auto**PBSCT**. These findings indicated minimal stromal damage with **Cyclo/TBI/TNI.**

Eighteen percent of the patients were preconditioned with **Bu/Cy**. The early peripheral blood parameter response numbers were low with only twenty percent of the allogeneic transplants generating an early **RMI** response, and fifty percent early **ANC** and **PLT** responses, ultimately sixty percent of them achieved a "12 month post transplant trilineage response". Although the stromal damage appeared to be greater than **Cyclo/TBI/TNI** the medium term success was more positive.

More than forty five percent of the patients produced Mononuclear Count (**MNC**) greater than the mean (6.12 $x10^8$/kg), approximately seventy percent of them generated a early peripheral blood parameter responses. Less than forty percent of them achieved a "12 month post transplant trilineage response". Although approximately sixty percent of the **PBSCT** generated early parameter responses only thirty percent of the **BMT** did, however in both transplants only thirty seven percent of the patients achieved a "12 month post transplant trilineage response". Of the **PBSCT**, about fifteen percent more of the allo**PBSCT** generated earlier parameter responses than the auto**PBSCT** although both were under fifty percent. Ultimately most of the patients with higher **MNC** relapsed. **MNC** greater than the mean generated an early response in about seventy percent of the patients.

Eighty percent of the patients were evaluated for **CFU-GM** counts. Approximately seventy percent with counts greater than the mean (26.10 $x10^4$/kg) generated early peripheral blood parameter responses. Eighty percent of the allogeneic transplants and fifty percent of the autologous transplants generated early responses, compared to seventy four percent of the **PBSCT** and nine percent of the **BMT**. Eighty three percent of the allo**PBSCT** generated early responses compared to fifty seven auto**PBSCT**, although the autologous groups achieved a higher "12 month post transplant trilineage response". The allogeneic transplants did however, generate a far higher percentage of early **PLT** responses than the other two parameters. Ultimately higher **CFU-GM** counts did predict

early peripheral blood parameter responses in more than fifty percent of the patients, particularly in the megakaryocytic line.

A Response Range was established for the first forty days, using the daily transplant data in the three peripheral blood parameters of the forty five patients who engrafted. The **RMI** range was found to be of value when monitoring "graft failures", particularly because every patient's **RMI** remained below the mean for the first forty days. Patients with **ANC** that rose above **2SD** at some point within the first forty days, devoid of infection, achieved a "12 month post transplant trilineage response". The **PLT** count was unreliable due to instability, which was due to fluctuations in platelet counts during the first forty days post transplant.

The "day 14" marrow biopsies revealed nothing that the "day 28" biopsies failed to. However, patients with **SAA** did benefit from the "day 14" bone marrow biopsies as evidence of "graft failure" was present in all biopsies. "Graft failure" was indicated prior to the "day 28" marrow on the three peripheral blood parameters.

The **RMI** proved to be the earliest indicator of **BMT** and **PBSCT** engraftment. The **RMI** was also the most reliable means of determining "graft failure". Patients who engrafted produced **RMI** values greater than 0.20 IU within seventeen days, failing that the patient faced imminent "graft failure". Early **RMI** response indicated minimal stromal damage due to preconditioning, unfortunately these values did not allow for medium term prediction in transplant success. More early **RMI** responses were seen in patients with **CFU-GM** counts greater than the established mean than higher **MNC**. Unfortunately neither the early **RMI** response in conjunction with higher **CFU-GM** or **MNC** predicted medium term transplant success. In cases where relying on "day 14" marrow biopsies to ensure trilineage recovery, it was found that the **RMI** along with the other two peripheral blood parameters were sufficient to establish engraftment.

CONGRESS PRESENTATIONS

1. **In Search of Precise and Accurate Reticulocyte Counts. Preliminary Results.**

   J M Blackbeard, Y Pillay.

   Presented at:

   - Medical Technology Congress - Cape Town. **1995**

2. **Setting Bit Maps for Flow Cytometric Reticulocyte Analysis.**

   J M Blackbeard, Y Pillay.

   Presented at:

   - Flow Cytometric Congress – Cape Town. **1996**

3. **Reticulocyte Stability Studies by Flow Cytometry.**

   J M Blackbeard, I Aronson.

   Presented at:

   - Medical Technology Congress – Bloemfontein. **1997**

4. **Reticulocyte Maturation Index as a Point of Engraftment in Post Bone Marrow Transplants. Preliminary Results.**

   J M Blackbeard, E H Lombard.

   Presented at:

   - Medical Technology Congress – Port Elizabeth. **1999**

     and

   - Research Day 2000 – **UCT** – Cape Town. **2000**

ABBREVIATIONS

- **ALG** = Anti Lymphocytic Globulin.
- **ALL** = Acute Lymphocytic Leukaemia.
- **AML** = Acute Myeloid Leukaemia.
- **ANC** = Absolute Neutrophil Count (The fraction of the leukocyte count used in study to predict granulocyte response post transplantation, reported in $x10^9/L$).
- Allo**BMT** = allogenic bone marrow transplant.
- Allo**PBSCT** = allogenic peripheral blood stem cell transplant.
- Auto**BMT** = autologous bone marrow transplant.
- Auto**PBSCT** = autologous peripheral blood stem cell transplant.
- **BCB** = Brilliant Cresyl Blue.
- **Biph** = Bi-phenotypic leukaemia.
- **BM** = Bone Marrow.
- **BMSCT** = Bone Marrow Stem Cell Transplant.
- **BMT** = Bone marrow transplant.
- **Bu** = Busulphan.
- **BFUe** = Burst Forming Unit erythrocytic (committed progenitors).
- **b.w.** = body weight.
- **CD34+** = Cluster Designation 34+.
- **CFUc** = Colony Forming Unit (committed progenitors).
- **CFUe** = Colony Forming Unit erythrocytic (committed progenitors).
- **CFU_{GEMM}** = Colony Forming Unit granulocytic, erythrocytic, macrocytic and megakaryocytic (pluripotent progenitors).
- **CFU-GM** = Colony Forming Unit Granulocytic and Monocytic.
- **CML** = Chronic Myelocytic Leukaemia.
- **CV** = Co efficient of Variance.
- **Cyclo** = Cyclophosphamide.
- **DNA** = Deoxribo Nucleic Acid.
- **FBC** = Full Blood Count.

- **FCM** = Flow Cytometric method.
- **G-CSF** = Granulocyte Colony Stimulating Factor.
- **GVHD** = Graft Versus Host Disease.
- **HFR** = High Fluorescent Rate.
- **HJ** = Howell Jolly Bodies.
- **HLA** = Human Lymphocytic Antigen.
- **IRF** = Immature Reticulocyte Fraction.
- **LFR** = Low Fluorescent Rate.
- **LL** = Lymphocytic Lymphoma.
- **MCR** = Mean Channel Rate.
- **Mel** = Melphalan.
- **MFI** = Mean Fluorescent Index.
- **MFR** = Medium Fluorescent Rate.
- **MNC** = Mononuclear count (The number of harvested mononuclear cells that the patient receives at time of transplant, reported in x 10^8/ nucleated cells received per kg body weight).
- **NHL** = Non Hodgkins Lymphoma.
- **PLT** = Platelet Count (The number of platelets used in the study to predict the platelet response post transplantation, reported in x10^9/L).
- **PB** = Peripheral Blood.
- **PBSCT** = Peripheral Blood Stem Cell Transplant.
- **POI** = Point of Infliction.
- **RBC** = Red Cell Count.
- **RET** = Total Reticulocyte Count.
- **RET%** = Total Reticulocyte Count Percentage.
- **RMI** = Reticulocyte Maturation Index (The number of high fluorescing reticulocytes counted divided by the total number of reticulocytes counted, reported in IU).
- **RNA** = Ribo Nucleic Acid.
- **RT** = Room Temperature.
- **SAA** = Severe Aplastic Anaemia.

- **Stem** = Stem Cell Leukaemia.
- **TIBC** = Total Iron Binding Capacity.
- **TBI** = Total Body Irradiation.
- **TNI** = Total Nodule Irradiation.
- **TO** = Thiozole Orange.
- **VRET%** = Visually Detected Reticulocye %.
- **WBC** = White Blood Count.

TABLE OF CONTENTS

Declaration.....i
Dedication.....ii
Acknowledgement.....iii
Abstract.....v
Congress Presentations.....x
Abbreviations.....xi
Figures.....xxx
Graphs.....xxxii

CHAPTER 1 Introduction.....1

1.1. History of Reticulocyte Measurements.....1
1.2. The Importance of the Study.....4
1.3. The Problem Statement.....5
1.4. Delimitations.....6
1.5. Assumptions.....7

CHAPTER 2 Literature Review.....8

2.1. Introduction.....8

2.2. Establishment of Automated Methods.....8
2.2.1. Thiozole Orange (**TO**) as a dye.....9

2.2.2. The Co Efficient of Variance (CV).....10
2.2.3. Reproducibility and Stability.....10
2.2.4. Linearity.....10
2.2.5. Incubation and Storage Time.....11
2.2.6. Batch Precision.....11
2.2.7. Comparison of Automated and Manual Reticulocyte Counts.....11
2.2.8. Instrument Precision.....12

2.3. Indicators of Aplasia.....12

2.4. Criteria for Engraftment.....13

2.5. Indicators of Engraftment.....13
2.5.1. Reticulocyte Count as an indicator of Engraftment.....13
2.5.2. Mean Fluorescent Index (**MFI**) as an Indication of Engraftment.....15
2.5.3. High Fluorescent Rate (**HFR**) as an Indicator for Engraftment.....16
2.5.4. High Fluorescent Rate plus Medium Fluorescent Rate (**HFR+MFR**) as an Indicator for Engraftment.....18
2.5.5. Reticulocyte Maturation Index (**RMI**) as an Indicator for Engraftment.....18
2.5.6. Discussion on Indicators of Engraftment.....20

2.6. Haemopoietic Progenitors.....23

2.7. Graft Failures.....25

2.8. Early Bone Marrow Biopsies.....26

2.9. Long Term Response.....27

CHAPTER 3 Materials and Methods.....28

3.1. The Flow Cytometer.....28
3.1.1. Principle.....28
3.1.2. Operation.....28

3.2. Instrumentation.....31
3.2.1. Instrument Protocol.....31
3.2.2. Establishment of the Reticulocyte Maturation Index (**RMI**).....33
3.2.3. The Control Group.....34

3.3. Methodology.....34
3.3.1. Reagent and Controls.....34
3.3.2. The Method.....34

3.4. The Data Collection.....34
3.4.1. The Primary Data Collection.....34
3.4.2. The Secondary Data Collection.....34
3.4.3. The Population Sample.....35
3.4.4. Statistical Analysis.....35

CHAPTER 4 Results.....36

4.1. Introduction.....36
4.1.1. Sample Population.....38
4.1.2. The Mean and Median Response Time for Reticulocyte Maturation Index (**RMI**), Absolute Neutrophil Count (**ANC**) and Platelet Count (**PLT**).....39

4.1.3. Point of Response for Reticulocyte Maturation Index (**RMI**), Absolute Neutrophil Count (**ANC**) and Platelet Count (**PLT**).....40
4.1.4. Preconditioning.....42
4.1.5. Mononuclear Count (**MNC**), Colony Forming Unit-Granulocytic Monocytic Count (**CFU-GM**) and Cluster Designation 34+ (**CD34+**) Counts.....43
4.1.6. Expected Response Range.....44
4.1.7. Medium Term Mean and Median Counts.....46

4.2. Comparison of Transplants.....49
4.2.1. The Reticulocyte Maturation Index (**RMI**) Response.....49
4.2.1.1. The Mean and Median Response Time for the Reticulocyte Maturation Index (**RMI**) on Transplant Patients.....49
4.2.1.2. The Point of Response for the Reticulocyte Maturation Index (**RMI**) on Transplant Patients.....50
4.2.2. The Absolute Neutrophil Count (**ANC**) Response.....51
4.2.2.1. The Mean and Median Response Time for the Absolute Neutrophil Count (**ANC**) on Transplant Patients.....51
4.2.2.2. The Point of Response for the Absolute Neutrophil Count (**ANC**) on Transplant Patients.....52
4.2.3. The Platelet Count (**PLT**) Response.....53
4.2.3.1. The Mean and Median Response Time for the Platelet Count (**PLT**) on Transplant Patients.....53
4.2.3.2. The Point of Response for the Platelet Count (**PLT**) on Transplant Patients.....54
4.2.4. Preconditioning in Transplant Patients.....55
4.2.5. Mononuclear Count (**MNC**), Colony Forming Unit – Granulocytic Monocytic (**CFU-GM**) and Cluster Designation 34+ Counts (**CD34+**) in Transplant Patients.....57
4.2.6. Response Range for Transplant Patients.....58
4.2.6.1. Reticulocyte Maturation Index (**RMI**).....59
4.2.6.2. Absolute Neutrophil Count (**ANC**).....59

4.2.6.3. Platelet Count (**PLT**).....59
4.2.7. "Day 14" Bone Marrow Biopsy Results on Transplant Patients.....59

4.3. Comparison of Allogenic and Autologous Transplants.....60
4.3.1. The Reticulocyte Maturation Index (**RMI**) Response.....60
4.3.1.1. The Mean and Median Response Time for the Reticulocyte Maturation Index (**RMI**) between Allogenic and Autologous Transplant Patients.....60
4.3.1.2. The Point of Response for the Reticulocyte Maturation Index (**RMI**) on Allogenic and Autologous Transplant Patients.....62
4.3.2. The Absolute Neutrophil Count (**ANC**) Response.....64
4.3.2.1. The Mean and Median Response Time for the Absolute Neutrophil Count (**ANC**) between Allogenic and Autologous Transplant Patients.....64
4.2.2.2. The Point of Response for the Absolute Neutrophil Count (**ANC**) Between Allogenic and Autologous Transplant Patients.....66
4.3.3. The Platelet Count (**PLT**) Response.....68
4.3.3.1. The Mean and Median Response Time for the Platelet Count (**PLT**) between Allogenic and Autologous Transplant Patients.....68
4.3.3.2. The Point of Response for the Platelet Count (**PLT**) Between Allogenic and Autologous Transplant Patients.....70
4.3.4. Preconditioning in Allogenic and Autologous Transplant Patients.....72
4.3.5. Mononuclear Count (**MNC**), Colony Forming Unit – Granulocytic Monocytic (**CFU-GM**) and Cluster Designation 34+ Counts (**CD34+**) in Allogenic and Autologous Transplant Patients.....73
4.3.6. Response Range for Allogenic and Autologous Transplant Patients.....75
4.3.6.1. Reticulocyte Maturation Index (**RMI**).....75
4.3.6.2. Absolute Neutrophil Count (**ANC**).....76
4.3.6.3. Platelet Count (**PLT**).....76
4.3.7. "Day 14" Bone Marrow Biopsy Results on Allogenic and Autologous Transplant Patients.....76

4.4. Comparison of Peripheral Blood Stem Cell Transplant (**PBSCT**) and Bone Marrow Transplant (**BMT**) Patients.....77
4.4.1. The Reticulocyte Maturation Index (**RMI**) Response.....77
4.4.1.1. The Mean and Median Response Time for the Reticulocyte Maturation Index (**RMI**) between Peripheral Blood Stem Cell Transplant (**PBSCT**) and Bone Marrow Transplant (**BMT**) Patients.....77
4.4.1.2. The Point of Response for (**RMI**) between Peripheral Blood Stem Cell Transplant (**PBSCT**) and Bone Marrow Transplant (**BMT**) Patients.....78
4.4.2. The Absolute Neutrophil Count (**ANC**) Response.....80
4.4.2.1. The Mean and Median Response Time for the Absolute Neutrophil Count (**ANC**) between Peripheral Blood Stem Cell transplant (**PBSCT**) and Bone Marrow Transplant (**BMT**) Patients.....80
4.4.2.2. The Point of Response for the Absolute Neutrophil Count (**ANC**) between Peripheral Blood Stem Cell Transplant (**PBSCT**) and Bone Marrow Transplant (**BMT**) Patients.....82
4.4.3. The Platelet Count (**PLT**) Response.....84
4.4.3.1. The Mean and Median Response Time for the Platelet Count (**PLT**) Between Peripheral Blood Stem Cell Transplant (**PBSCT**) and Bone Marrow Transplant (**BMT**) Patients.....84
4.4.3.2. The Point of Response for the Platelet Count (**PLT**) between Peripheral Blood Stem Cell Transplant (**PBSCT**) and Bone Marrow Transplant (**BMT**) Patients.....85
4.4.4. Preconditioning in Peripheral Blood Stem Cell Transplant (**PBSCT**) and Bone Marrow Transplant (**BMT**) Patients.....87
4.4.5. Mononuclear Count (**MNC**), Colony Forming Unit – Granulocytic Monocytic (**CFU-GM**) and Cluster Designation CD34+ Counts (**CD34+**) in Peripheral Blood Stem Cell Transplant (**PBSCT**) and Bone Marrow (**BMT**) Patients.....89
4.4.6. Response Range for Peripheral Blood Stem Cell Transplant (**PBSCT**) and Bone Marrow Transplant (**BMT**) Patients.....90

4.4.6.1. Reticulocyte Maturation Index (**RMI**).....90
4.4.6.2. Absolute Neutrophil Count (**ANC**).....91
4.4.6.3. Platelet Count (**PLT**).....91
4.4.7. "Day 14" Bone Marrow Biopsy results on Peripheral Blood Stem Cell Transplant (**PBSCT**) and Bone Marrow Transplant (**BMT**) Patients.....91

4.5. Comparison of allogenic Peripheral Blood Stem Cell Transplant (allo**PBSCT**) and autologous Peripheral Blood Stem Cell Transplant (auto**PBSCT**) Patients.....92
4.5.1. The Reticulocyte Maturation Index (**RMI**) Response.....92
4.5.1.1. The Mean and Median Response Time for the Reticulocyte Maturation Index (**RMI**) between allogenic Peripheral Blood Stem Cell Transplant (allo**PBSCT**) and autologous Peripheral Blood Stem Cell Transplant (auto**PBSCT**) Patients.....92
4.5.1.2. The Point of Response for The Reticulocyte Maturation Index (**RMI**) between allogenic Peripheral Blood Stem Cell Transplant (allo**PBSCT**) and autologous Peripheral Blood Stem Cell Transplant (auto**PBSCT**) patients.....94
4.5.2. The Absolute Neutrophil Count (**ANC**) Response.....96
4.5.2.1. The Mean and the Median Response Time for the Absolute Neutrophil Count (**ANC**) between allogenic Peripheral Blood Stem Cell Transplant (allo**PBSCT**) and autologous Peripheral Blood Stem Cell Transplant (auto**PBSCT**) Patients.....96
4.5.2.2. The Point of Response for the Absolute Neutrophil Count (**ANC**) between allogenic Peripheral Blood Stem Cell Transplant (allo**PBSCT**) and autologous Peripheral Blood Stem Cell Transplant (auto**PBSCT**) Patients.....98
4.5.3. The Platelet Count (**PLT**) Response.....100
4.5.3.1. The Mean and Median Response Time for The Platelet Count (**PLT**) between allogenic Peripheral Blood Stem Cell Transplant (allo**PBSCT**)

and autologous Peripheral Blood Stem Cell Transplant (auto**PBSCT**) Patients.....**100**

4.5.3.2. The Point of Response for the Platelet Count (**PLT**) between allogenic Peripheral Blood Stem Cell Transplant (allo**PBSCT**) and autologous Peripheral Blood Stem Cell Transplant (auto**PBSCT**) Patients.....**102**

4.5.4. Preconditioning in allogenic Peripheral Blood Stem Cell Transplant (allo**PBSCT**) and autologous Peripheral Blood Stem Cell Transplant (auto**PBSCT**) Patients.....**104**

4.5.5. Mononuclear Count (**MNC**), Colony Forming Unit – Granulocytic Monocytic (**CFU-GM**) and Cluster Designation 34+ Counts (**CD34+**) in allogenic Peripheral Blood Stem Cell Transplant (allo**PBSCT**) and autologous Peripheral Blood Stem Cell Transplant (auto**PBSCT**) Patients.....**106**

4.5.6. Response Range for allogenic Peripheral Blood Stem Cell Transplant (allo**PBSCT**) and autologous Peripheral Blood Stem Cell Transplant (auto**PBSCT**) Patients.....**108**

4.5.6.1. Reticulocyte Maturation Index (**RMI**).....**108**

4.5.6.2. Absolute Neutrophil Count (**ANC**).....**108**

4.5.6.3. Platelet Count (**PLT**).....**108**

4.5.7. "Day 14" Bone Marrow Biopsy Results on allogenic Peripheral Blood Stem Cell Transplant (allo**PBSCT**) and autologous Peripheral Blood Stem Cell Transplant (auto**PBSCT**) Patients.....**108**

4.6. Acute Myeloid Leukaemia allogenic Peripheral Blood Stem Cell Transplant (**AML** allo**PBSCT**) **P**atients.....**109**

4.6.1. The Reticulocyte Maturation Index (**RMI**) Response.....**109**

4.6.1.1. The Mean and Median Response Time for the Reticulocyte Maturation Index (**RMI**) in Acute Myeloid Leukaemia allogenic Peripheral Blood Stem Cell Transplant (**AML** allo**PBSCT**) Patients.....**109**

4.6.1.2. The Point of Response for the Reticulocyte Maturation Index (**RMI**) in Acute Myeloid Leukaemia allogenic Peripheral Blood Stem Cell Transplant (**AML** allo**PBSCT**) Patients.....**110**
4.6.2. The Absolute Neutrophil Count (**ANC**) Response.....**111**
4.6.2.1. The Mean and Median Response Time for the Absolute Neutrophil Count (**ANC**) in Acute Myeloid Leukaemia allogenic Peripheral Blood Stem Cell Transplant (**AML** allo**PBSCT**) Patients.....**111**
4.6.2.2. The Point of Response for the Absolute Neutrophil Count (**ANC**) in Acute Myeloid Leukaemia allogenic Peripheral Blood Stem Cell Transplant (**AML** allo**PBSCT**) Patients.....**112**
4.6.3. The Platelet Count (**PLT**) Response.....**113**
4.6.3.1. The Mean and Median Response Time for the Platelet Count (**PLT**) in Acute Myeloid Leukaemia allogenic Peripheral Blood Stem Cell Transplant (**AML** allo**PBSCT**) Patients.....**113**
4.6.3.2. The Point of Response for the Platelet Count (**PLT**) in Acute Myeloid Leukaemia allogenic Peripheral Blood Stem Cell Transplant (**AML** allo**PBSCT**) Patients.....**114**
4.6.4. Preconditioning in Acute Myeloid Leukaemia allogenic Peripheral Blood Stem Cell Transplant (**AML** allo**PBSCT**) Patients.....**114**
4.6.5. Mononuclear Count (**MNC**), Colony Forming Unit – Granulocytic Monocytic (**CFU-GM**) and Cluster Designation 34+ Counts (**CD34+**) in Acute Myeloid Leukaemia allogenic Peripheral Blood Stem Cell Transplant (**AML** allo**PBSCT**) Patients.....**115**
4.6.6. Response Range for Acute Myeloid Leukaemia allogenic Peripheral Blood Stem Cell Transplant (**AML** allo**PBSCT**) Patients.....**116**
4.6.6.1. Reticulocyte Maturation Index (**RMI**).....**116**
4.6.6.2. Absolute Neutrophil Count (**ANC**).....**116**
4.6.6.3. Platelet Count (**PLT**).....**116**
4.6.7. "Day 14" Bone Marrow Biopsy Results on Acute Myeloid Leukaemia allogenic Peripheral Blood Stem Cell Transplant (**AML** allo**PBSCT**) Patients.....**116**

4.7. Chronic Myeloid Leukaemia allogenic Peripheral Blood Stem Cell Transplant (**CML** allo**PBSCT**) Patients.....**117**
4.7.1. The Reticulocyte Maturation Index (**RMI**) Response.....**117**
4.7.1.1. The Mean and Median Response Time for the Reticulocyte Maturation Index (**RMI**) in Chronic Myeloid Leukaemia allogenic Peripheral Stem Cell Transplant (**CML** .allo**PBSCT**) Patients.....**117**
4.7.1.2. The Point of Response for Reticulocyte Maturation Index (**RMI**) in Chronic Myeloid Leukaemia allogenic Peripheral Blood Stem Cell Transplants (**CML** allo**PBSCT**) Patients.....**118**
4.7.2. The Absolute Neutrophil Count (**ANC**) Response.....**119**
4.7.2.1. The Mean and Median Response Time for the Absolute Neutrophil Count (**ANC**) in Chronic Myeloid Leukaemia allogenic Peripheral Blood Stem Cell Transplant (**CML** allo**PBSCT**) Patients.....**119**
4.7.2.2. The Point of Response for the Absolute Neutrophil Count (**ANC**) in Chronic Myeloid Leukaemia allogenic Peripheral Blood Stem Cell Transplant (**CML** allo**PBSCT**) Patients.....**120**
4.7.3. The Platelet Count (**PLT**) Response.....**121**
4.7.3.1. The Mean and Median Response Time for the Platelet Count (**PLT**) in Chronic Myeloid Leukaemia allogenic Peripheral Blood Stem Cell Transplant (**CML** allo**PBSCT**) Patients.....**121**
4.7.3.2. The Point of Response for the Platelet Count (**PLT**) in Chronic Myeloid Leukaemia allogenic Peripheral Blood Stem Cell Transplant (**CML** allo**PBSCT**) Patients.....**122**
4.7.4. Preconditioning in Chronic Myeloid Leukaemia allogenic Peripheral Blood Stem Cell Transplant (**CML** allo**PBSCT**) Patients.....**122**
4.7.5. Mononuclear Count (**MNC**), Colony Forming Unit – Granulocytic Monocytic (**CFU-GM**) and Cluster Designation 34+ Counts (**CD34+**) in Chronic Myeloid Leukaemia allogenic Peripheral Blood Stem Cell Transplant (**CML** allo**PBSCT**) Patients.....**123**
4.7.6. Response Range of Chronic Myeloid Leukaemia allogenic Peripheral Blood Stem Cell Transplant (**CML** allo**PBSCT**) Patients.....**124**

4.7.6.1. Reticulocyte Maturation Index (**RMI**).....124
4.7.6.2. Absolute Neutrophil Count (**ANC**).....124
4.7.6.3. Platelet Count (**PLT**).....124
4.7.7. "Day 14" Bone Marrow Biopsy Results on the Chronic Myeloid Leukaemia allogenic Peripheral Blood Stem Cell Transplant (**CML alloPBSCT**) Patients.....124

4.8. Acute Myeloid Leukaemia autologous Peripheral Blood Stem Cell Transplant (**AML autoPBSCT**) Patients.....125
4.8.1. The Reticulocyte Maturation Index (**RMI**) Response.....125
4.8.1.1. The Mean and Median Response Time for the Reticulocyte Maturation Index (**RMI**) in Acute Myeloid Leukaemia autologous Peripheral Blood Stem Cell Transplant (**AML autoPBSCT**) Patients.....125
4.8.1.2. The Point of Response for the Reticulocyte Maturation Index (**RMI**) in Acute Myeloid Leukaemia autologous Peripheral Blood Stem Cell Transplant (**AML autoPBSCT**) Patients.....126
4.8.2. The Absolute Neutrophil Count (**ANC**) Response.....127
4.8.2.1. The Mean and Median Response Time for the Absolute Neutrophil Count (**ANC**) in Acute Myeloid Leukaemia autologous Peripheral Blood Stem Cell Transplant (**AML autoPBSCT**) Patients.....127
4.8.2.2. The Point of Response for the Absolute Neutrophil Count (**ANC**) in Acute Myeloid Leukaemia autologous Peripheral Blood Stem Cell Transplant (**AML autoPBSCT**) Patients.....128
4.8.3. The Platelet Count (**PLT**) Response.....129
4.8.3.1. The Mean and Median Response Time for the Platelet Count (**PLT**) in Acute Myeloid Leukaemia autologous Peripheral Blood Stem Cell Transplant (**AML autoPBSCT**) Patients.....129
4.8.3.2. The Point of Response for the Platelet Count (**PLT**) in Acute Myeloid Leukaemia autologous Peripheral Blood Stem Cell Transplant (**AML autoPBSCT**) Patients.....130

4.8.4. Preconditioning in Acute Myeloid Leukaemia autologous Peripheral Blood Stem Cell Transplant (**AML** auto**PBSCT**) Patients.....**131**

4.8.5. Mononuclear Count (**MNC**), Colony Forming Unit – Granulocytic Monocytic (**CFU-GM**) and Cluster Designation 34+ Counts (**CD34+**) in Acute Myeloid Leukaemia autologous Peripheral Blood Stem Cell Transplant (**AML** auto**PBSCT**) Patients.....**132**

4.8.6. Response Range for Acute Myeloid Leukaemia autologous Peripheral Blood Stem Cell Transplant (**AML** auto**PBSCT**) Patients.....**133**

4.8.6.1. Reticulocyte Maturation Index (**RMI**).....**133**

4.8.6.2. Absolute Neutrophil Count (**ANC**).....**133**

4.8.6.3. Platelet Count (**PLT**).....**133**

4.8.7. "Day 14" Bone Marrow Biopsy results on Acute Myeloid Leukaemia autologous Peripheral Blood Stem Cell Transplant (**AML** auto**PBSCT**) Patients.....**133**

4.9. "12 month post transplant trilineage response" patients.....**134**

4.9.1. The Reticulocyte Maturation Index (**RMI**) Response.....**134**

4.9.1.1. The Mean and Median Response Time for the Reticulocyte Maturation Index (**RMI**) in the "12 month post transplant trilineage response" Patients.....**134**

4.9.1.2. The Point of Response for the Reticulocyte Maturation Index (**RMI**) in the "12 month post transplant trilineage response" Patients.....**135**

4.9.2. The Absolute Neutrophil Count (**ANC**) Response.....**136**

4.9.2.1. The Mean and the Median Response Time for the Absolute Neutrophil Count (**ANC**) in the "12 month post transplant trilineage response" Patients.....**136**

4.9.2.2. The Point of Response for the Absolute Neutrophil Count (**ANC**) in the "12 month post transplant trilineage response" Patients.....**137**

4.9.3. The Platelet Count (**PLT**) Response.....**138**

4.9.3.1. The Mean and the Median Response Time for the Platelet Count (**PLT**) in the "12 month post transplant trilineage response" Patients.....**138**

4.9.3.2. The Point of Response for Platelet Count (**PLT**) in the "12 month post transplant trilineage response" Patients.....139
4.9.4. Preconditioning in the "12 month post transplant trilineage response" Patients.....140
4.9.5. Mononuclear Count (**MNC**), Colony Forming Unit – Granulocytic Monocytic (**CFU-GM**) and Cluster Desigantion 34+ Counts (**CD34+**) in the "12 month post transplant trilineage response" Patients.....141
4.9.6. Response Range for the "12 month post transplant trilineage response" Patients.....142
4.9.6.1. Reticulocyte Maturation Index (**RMI**).....142
4.9.6.2. Absolute Neutrophil Count (**ANC**).....142
4.9.6.3. Platelet Count (**PLT**).....142
4.9.7. "Day 14" Bone Marrow Biopsy results in the "12 month post transplant trilineage response" Patients.....142

4.10. The Graft Failures.....143
4.10.1. The first Severe Aplastic Anaemia allogenic Bone Marrow Transplant (**SAA alloBMT**).....143
4.10.1.1. Reticulocyte Maturation Index (**RMI**) Response Time.....143
4.10.1.2. Reticulocyte Maturation Index (**RMI**) Point of Response.....143
4.10.1.3. Absolute Neutrophil Count (**ANC**) Response Time.....143
4.10.1.4. Absolute Neutrophil Count (**ANC**) Point of Response.....143
4.10.1.5. Platelet Count (**PLT**) Response Time.....144
4.10.1.6. Platelet Count (**PLT**) Point of Response.....144
4.10.1.7. Preconditioning.....144
4.10.1.8. Mononuclear Count (**MNC**) and Colony forming Unit (**CFU**) Counts.....144
4.10.1.9. The Response Range.....145
4.10.1.10. "Day 14" Bone Marrow Biopsy.....146

4.10.2. The Second Severe Aplastic Anaemia allogenic Bone Marrow Transplant (**SAA alloBMT**).....146

4.10.2.1. Reticulocyte Maturation Index (**RMI**) Response Time.....146
4.10.2.2. Reticulocyte Maturation Index (**RMI**) Point of Response.....146
4.10.2.3. Absolute Neutrophil Count (**ANC**) Response Time.....147
4.10.2.4. Absolute Neutrophil Count (**ANC**) Point of Response.....147
4.10.2.5. Platelet Count (**PLT**) Response Time.....147
4.10.2.6. Platelet Count (**PLT**) Point of Response.....147
4.10.2.7. Preconditioning.....148
4.10.2.8. Mononuclear Count (**MNC**) and Colony forming Unit (**CFU**) Counts.....148
4.10.2.9. Response Range.....149
4.10.2.10. "Day 14" Bone Marrow Biopsy.....148

4.10.3. An Acute Myeloid Leukaemia autologous Peripheral Blood Stem Cell Transplant (**AML autoPBSCT**).....149
4.10.3.1. Reticulocyte Maturation Index (**RMI**) Response Time.....149
4.10.3.2. Reticulocyte Maturation Index (**RMI**) Point of Response.....149
4.10.3.3. Absolute Neutrophil Count (**ANC**) Response Time.....149
4.10.3.4. Absolute Neutrophil Count (**ANC**) Point of Response.....150
4.10.3.5. Platelet Count (**PLT**) Response Time.....150
4.10.3.6. Platelet Count (**PLT**) Point of Response.....150
4.10.3.7. Preconditioning.....150
4.10.3.8. Mononuclear Count (**MNC**) and Colony Forming Unit (**CFU**) Counts.....150
4.10.3.9. Response Range.....151
4.10.3.10. "Day 14" Bone Marrow Biopsy.....151

4.10.4. A Severe Aplastic Anaemia allogenic Peripheral Blood Stem Cell Transplant (**SAA alloPBSCT**).....151
4.10.4.1. Reticulocyte Maturation Index (**RMI**) Response Time.....151
4.10.4.2. Reticulocyte Maturation Index (**RMI**) Point of Response.....152
4.10.4.3. Absolute Neutrophil Count (**ANC**) Response Time.....152
4.10.4.4. Absolute Neutrophil Count (**ANC**) Point of Response.....152
4.10.4.5. Platelet Count (**PLT**) Response Time.....152

4.10.4.6. Platelet Count (**PLT**) Point of Response.....153
4.10.4.7. Preconditioning.....153
4.10.4.8. Mononuclear Count (**MNC**) and Colony Forming Unit (**CFU**) Count.....153
4.10.4.9. Response Range.....154
4.10.4.10. "Day 14" Bone Marrow Biopsy.....154

CHAPTER 5 Discussion.....155

5.1. Introduction.....155

5.2. Reticulocyte Maturation Index (**RMI**) as an indicator for Reticulocyte Response.....157

5.3. The Mean Response Time.....158
5.3.1 Reticulocyte Maturation Index (**RMI**).....158
5.3.2 Absolute Neutrophil Count (**ANC**).....161
5.3.3 Platelet Count (**PLT**).....163

5.4. Point of Response.....165
5.4.1. Reticulocyte Maturation Index (**RMI**).....165
5.4.2. Absolute Neutrophil Count (**ANC**).....166
5.4.3. Platelet Count (**PLT**).....168

5.5. Preconditioning.....170

5.6. Mononuclear Count (**MNC**).....172

5.7. Colony Forming Unit – Granulocytic Monocytic (**CFU-GM**).....175

5.8. Cluster Designation 34+ (**CD34+**).....**178**

5.9. Response Range.....**178**
5.9.1 Reticulocyte Maturation Index (**RMI**).....**177**
5.9.2. Absolute Neutrophil Count (**ANC**).....**179**
5.9.3. Platelet Count (**PLT**).....**180**

5.10. "Day 14" Bone Marrow Biopsy.....**180**

CHAPTER 6 Conclusion.....183

FURTHER STUDIES.....187

BIBLIOGRAPHY.....188

FIGURES

Figure 1: Internal view of a flow cytometer.....**30**

Figure 2: A print out of reticulocyte analysis on an **EPICS Profile II** flow cytometer...**32**

Figure 3: Channels and established normal range for the Coulter **EPICS Profile II** flow cytometer at Groote Schuur Hospital.....**33**

Figure 4: Transplant Patients.....**38**

Figure 5: Mean Response Time and range for Reticulocyte Maturation Index (**RMI**), Absolute Neutrophil Count (**ANC**) and Platelet Count (**PLT**).....**39**

Figure 6: Median Response Time and range for Reticulocyte Maturation Index (**RMI**), Absolute Neutrophil Count (**ANC**) and Platelet Count (**PLT**).....**40**

Figure 7: Indication of days at which 50%, 75% and 95% of the patients reached their Point of Response.....**41**

Figure 8: All the Mononuclear Counts (**MNC**), Colony Forming Unit Granulocytic and Monocytic (**CFU-GM**) and Cluster Designation 34+ (**CD+ 34**) means for the transplant patients.....**44**

Figure 9: Mean of all forty five patients with Two Standard Deviation (**2SD**).....**45**

Figure 10: Mean Reticulocyte Maturation Index (**RMI**) results for transplants at day forty, three months, four months, five months and six months and percentage decrease.....**46**

Figure 11: Median Reticulocyte Maturation Index (**RMI**) results for transplants at day forty, three months, four months, five months and six months and percentage decrease.....**46**

Figure 12: Mean Absolute Neutrophil Count (**ANC**) results for transplants at day forty, three months, four months, five months and six months and percentage increase. * Indicates a decrease in value from forty days to six months.....**47**

Figure 13: Median Absolute Neutrophil Count (**ANC**) results for transplants at day forty, three months, four months, five months and six months and percentage increase. * Indicates a decrease in value from forty days to six months.....**47**

Figure 14: Mean Platelet Count (**PLT**) results for transplants at day forty, three months, four months, five months and six months and percentage increase.....**48**

Figure15: Median Platelet Count (**PLT**) results for transplants at day forty, three months, four months, five months and six months and percentage increase.....**48**

GRAPHS

Graph 1: The mean and median **RMI** Response Time, in days, for forty five transplant patients.....**49**

Graph 2: The **RMI** Point of Response, in days, for forty five transplant patients, 50%, 75% and 95% are highlighted.....**50**

Graph 3: The mean and median **ANC** Response Time, in days, for forty four transplant patients.....**51**

Graph 4: The **ANC** Point of Response, in days, for forty two patients transplant patients, 50%, 75% and 95% are highlighted.....**52**

Graph 5: The mean and median **PLT** Response Time, in days, for forty three transplant patients.....**53**

Graph 6: The **PLT** Point of Response, in days, for forty two transplant patients, 50%, 75% and 95% are highlighted.....**54**

Graph 7: The mean **RMI** Response Time, in days, for Thirty two allogenic and thirteen autologous transplant patients.....**60**

Graph 8: The **RMI** Point of Response, in days, for thirty two allogenic transplant patients, 50%, 75% and 95% are highlighted.....**62**

Graph 9: The **RMI** Point of Response, in days, for thirteen autologous transplant patients, 50%, 75% and 95% are highlighted.....**63**

Graph 10: The mean **ANC** Response Time, in days, for thirty two allogenic and thirteen autologous transplant patients.....**64**

Graph 11: The **ANC** Point of Response, in days, for thirty two allogenic transplant patients, 50%, 75% and 95% are highlighted.....**66**

Graph 12: The **ANC** Point of Response, in days, for thirteen autologous transplant patients, 50%, 75% and 95% are highlighted.....**67**

Graph 13: The mean **PLT** Response Time, in days, for thirty two allogenic and thirteen autologous transplant patients.....**68**

Graph 14: The **PLT** Point of Response, in days for thirty allogenic transplant patients, 50%, 75% and 95% are highlighted.....**70**

Graph 15: The **PLT** Point of Response, in days, for twelve autologous transplant patients, 50%, 75% and 95% are highlighted.....**71**

Graph 16: The mean **RMI** Response Time, in days, for thirty five **PBSCT** and ten **BMT** patients.....**77**

Graph 17: The **RMI** Point of Response, in days, for thirty five **PBSCT** patients, 50%, 75% and 95% are highlighted.....**78**

Graph 18: The **RMI** Point of Response, in days, for ten **BMT** patients, 50%, 75%, and 95% are highlighted.....**79**

Graph 19: The mean **ANC** Response Time, in days, for thirty five **PBSCT** patients and ten **BMT** patients.....**80**

Graph 20: The **ANC** Point of Response, in days, for thirty two **PBSCT** patients, 50%, 75%, and 95% are highlighted.....**82**

Graph 21: The **ANC** Point of Response, in days, for ten **BMT** patients, 50%, 75% and 95% are highlighted.....**83**

Graph 22: The mean **PLT** Response Time, in days, for thirty five **PBSCT** patients and eight **BMT** patients.....**84**

Graph 23: The **PLT** Point of Response, in days, for thirty five **PBSCT** patients, 50%, 75% and 95 % are highlighted.....**85**

Graph 24: The **PLT** Point of Response, in days, for eight **BMT** patients, 50%, 75% and 95% are highlighted.....**86**

Graph 25: The mean **RMI** Response Time, in days, for twenty two allo**PBSCT** patients and thirteen auto**PBSCT** patients.....**92**

Graph 26: The **RMI** Point of Response, in days, for twenty two allo**PBSCT** patients, 50%, 75% and 95% are highlighted.....**94**

Graph 27: The **RMI** Point of Response, in days, for thirteen auto**PBSCT** patients, 50%, 75% and 95% are highlighted.....**95**

Graph 28: The mean **ANC** Response Time, in days, for twenty two allo**PBSCT** patients and thirteen auto**PBSCT** patients.....**96**

Graph 29: The **ANC** Point of Response, in days, for twenty allo**PBSCT** patients, 50%, 75% and 95% are highlighted.....**97**

Graph 30: The **ANC** Point of Response, in days, for thirteen auto**PBSCT** patients, 50%, 75% and 95% are highlighted.....**99**

Graph 31: The mean **PLT** Response Time, in days, for twenty two allo**PBSCT** patients and thirteen auto**PBSCT** patients.....**100**

Graph 32: The **PLT** Point of Response, in days, for twenty one allo**PBSCT** patients, 50%, 75% and 95% are highlighted.....**102**

Graph 33: The **PLT** Point of Response, in days, for thirteen auto**PBSCT** patients, 50%, 75% and 95% are highlighted.....**103**

Graph 34: The mean and median **RMI** Response Time, in days, for eight **AML** allo**PBSCT** patients.....**109**

Graph 35: The **RMI** Point of Response, in days, for eight **AML** allo**PBSCT** patients, 50%, 75% and 95% are highlighted.....**110**

Graph 36: The mean and median **ANC** Response Time, in days, for eight **AML** allo**PBSCT** patients.....**111**

Graph 37: The **ANC** Point of Response, in days, for eight **AML** allo**PBSCT** patients, 50%, 75% and 95% are highlighted.....**112**

Graph 38: The mean and median **PLT** Response Time, in days, for eight **AML** allo**PBSCT** patients.....**113**

Graph 39: The **PLT** Point of Response, in days, eight **AML** allo**PBSCT** patients, 50%, 75% and 95% are highlighted.....**114**

Graph 40: The mean and median **RMI** Response Time, in days, for seven **CML** allo**PBSCT** patients.....117

Graph 41: The **RMI** Point of Response, in days, for seven **CML** allo**PBSCT** patients, 50%, 75% and 95% are highlighted.....118

Graph 42: The mean and median **ANC** Response Time, in days, for seven **CML** allo**PBSCT** patients.....119

Graph 43: The **ANC** Point of Response, in days, for seven **CML** allo**PBSCT** patients, 50%, 75% and 95% are highlighted.....120

Graph 44: The mean and median **PLT** Response Time, in days, for seven **CML** allo**PBSCT** patients.....121

Graph 45: The **PLT** Point of Response, in days, for seven **CML** allo**PBSCT** patients, 50%, 75% and 95% are highlighted.....122

Graph 46: The mean and median **RMI** Response Time, in days, for nine **AML** auto**PBSCT**) patients.....125

Graph 47: The Reticulocyte **RMI** Point of Response, in days, for nine **AML** auto**PBSCT** patients, 50%, 75% and 95% are highlighted.....126

Graph 48: The mean and median **ANC** Response Time in days, for nine **AML** auto**PBSCT** patients.....127

Graph 49: The **ANC**) Point of Response, in days, for nine **AML**. auto**PBSCT** patients, 50%, 75% and 95 % are highlighted.....128

Graph 50: The mean and median **PLT** Response Time, in days, for nine **AML** auto**PBSCT** patients.....**129**

Graph 51: The **PLT** Point of Response, in days, for nine **AML** auto**PBSCT** patients, 50%, 75% and 95% are highlighted.....**130**

Graph 52: The mean and median **RMI** Response Time, in days, for twenty two "12 month post transplant trilineage response" patients.....**134**

Graph 53: The **RMI** Point of Response, in days, for twenty two "12 month post transplant trilineage response" patients, 50%, 75% and 95% are highlighted.....**135**

Graph 54: The mean and median **ANC** Response Time, in days, for twenty two "12 month post transplant trilineage response" patients.....**136**

Graph 55: The **ANC** Point of Response, in days for twenty two "12 month post transplant trilineage response" patients, 50%, 75% and 95% are highlighted.....**137**

Graph 56: The mean and median **PLT** Response Time, in days, for twenty two "12 month post transplant trilineage response" patients.....**138**

Graph 57: All the **PLT** Point of Response, in days, for twenty two "12 month post transplant trilineage response" patients, 50%, 75% and 95% are highlighted.....**139**

CHAPTER 1

Introduction

1.1. History of Reticulocyte Measurements

The manual reticulocyte count has always been an inexpensive and easy approach for measuring bone marrow erythroid response particularly following stimulation or erythropoietic need (Cavill *et al*,1992 & 1993;Cline *et al*,1963;Ganzoni *et al*,1969;Hillman *et al*,1968 & 1969;Mel *et al*,1977;Perrotta *et al*,1972). The traditional manual supravital staining techniques have been the most commonly adopted methods in the past. Stains such as Azure B (Marshall *et al*,1976), New Methylene Blue (Brecher *et al*,1949;Deiss *et al*,1970), Brilliant Cresyl Blue (Dacie & Lewis, 1984) and Acridine Orange (Paul *et al*,1983;Vander *et al*,1963;Vaughan *et al*,1963) for fluorescent detection of reticulocytes were used. However, these manual methods are historically inaccurate due to badly prepared slides, they are subject to poor staining techniques, only a limited number of cells are counted and individual operator interpretation of reticulocyte definition is common (Brecher *et al*,1950;Koepke *et al*,1986;Peebles *et al*,1981).

Manual methods are also limited in regard to how much information can be presented. They only offer a Total Reticulocyte Percentage. If the red cell count is available then a further corrected count and an absolute count can be obtained. Heilmeyer *et al* (1932) attempted to create additional indices by introducing a more complex count. He graded reticulocytes by visually estimating the **RNA** content and scoring each cell individually. He classified four "Types" of reticulated cells, ranging from Type I to Type IV; Type I being the cell containing the least amount of **RNA**, to Type IV containing the most. The Heilmeyer classification gives a good indication of the maturity in a cell range. Unfortunately, the method is time consuming and prone to human variation (Crouch *et al*, 1985;Gilmer *et al*,1976).

With the introduction of automated reticulocyte counts we have managed to eliminate human errors and discrepancies. Flow cytometry particularly has the potential of being a superior method to manual counting techniques for several reasons. Automation allows for larger cell volumes to be counted. Instead of a hundred cells, we are able to count fifty thousand. Less statistical error is observed due to the larger number of cells analysed. It is faster than microscopic counting. More reproducible results are obtained, due to the fact that larger numbers are counted. Errors with supravital stains are eliminated. Particularly with New Methylene Blue, as it stains siderotic granules, leading to considerable errors in some clinical situations. Wedge spread blood films do not produce a uniform spread of blood cells, although spun films can produce a more homogeneous distribution of cells, resulting in inevitable statistical variations. Differences of greater than 30% **CV** can occur due to technologist variation (Lofsness *et al*,1994;Paterakis *et al*,1996;Tichelli *et al*,1990;Wells *et al*,1992).

Over the years numerous papers have been published to establish the best staining techniques for flow cytometric reticulocyte analysis. A variety of fluorescent stains bind with **RNA**, including Pyronin Y (Tanke *et al*, 1980 & 1983), Acridine Orange (Warren *et al*,1980), Propidium Iodide (Warren *et al*,1980), Cyanide dye 3.3'-dimethyloxacarbocyanide (Jaccobberger *et al*,1984), Thioflavine T (Metzger *et al*,1987;Sage *et al*,1983). Such flow cytometric staining methods offer the potential regarding greater precision, improved reproducibility, less subjectivity, and reduced technical effort, provided that such methods can successfully be integrated into the clinical haematology laboratory.

Since fluorescence intensity is directly proportional to the amount of cellular **RNA**, flow cytometric fluorescence measurements of reticulocytes provide an objective measurement of red cell maturity. Despite the theoretical advantages of flow cytometric fluorescent reticulocyte analysis, many of the reported techniques have limitations. The Pyronin Y technique requires fixation steps which cause it to be relatively time-consuming and thus, not cost-effective in the clinical laboratory environment (Tanke *et al*,1980 & 1983). The Acridine Orange method has the limitation of requiring thorough instrument cleansing

following use to avoid interference of the absorbed dye with subsequent measurements. Unfortunately there are still dye deposits even after thorough cleaning (Warren *et al*,1980). Additionally, Acridine Orange, Pyronin Y, and 3.3'-dimethyloxacarbocyanide all have low affinity to **RNA**, thus making resolution of the reticulocyte population relatively difficult (Warren *et al*,1980). The Thioflavine T method has the disadvantage of being time dependent thus being a potential source of error, and neither **RNA** nor **DNA** reach saturation with Thioflavine T (Metzger *et al*,1987;Sage *et al*,1983).

Various papers provide evidence that Thiazole Orange (**TO**) is the best dye to use (Carter *et al*,1989;Chin-Yee *et al*,1991). The fluorescent dye **TO** was first commercially available and evaluated in 1986 by Lee *et. al.* With a stable staining period of thirty to ninety minutes and a simple staining procedure, staining with **TO** is straightforward and requires small volumes of whole blood. **RNA** is not denatured upon binding and provides a more stable fluorescent signal. This facilitates a cost-effective method and the creation of additional reticulocyte parameters such as, Low Fluorescent Rate (**LFR**), Medium Fluorescent Rate (**MFR**) and High Fluorescent Rate (**HFR**) and a Reticulocyte Maturity Index (**RMI**) (Davis *et al*,1992 & 1993).

The advantage of **TO** analysis is that the staining procedure is more straightforward than Thioflavine T, the analysis is simpler than Pyronin Y or (3.3'-dimethyloxacarbocyanide) and ($DiOC_3$). Thus several advantages have been gained over previously used dyes in flow cytometry.

TO gives excellent fluorescent excitation with commonly used 488-nm argon laser flow cytometers with a high "quantum yield", making reticulocyte identification simpler and more accurate. **TO** offers greater simplicity in both staining and analysis. It also provides greater precision than New Methylene Blue owing to its intrinsic advantages of instrumental analysis over manual analysis. A potential disadvantage of **TO** is that the staining is indiscriminate; **DNA** and **RNA** are both stained, but it has been shown that leukocytes and platelets do not interfere with analysis.

Autofluorescence of red cells can be seen as a second red blood cell cluster. Leukocytes and nucleated red blood cells can be identified as further clusters on the cytogram. These are eliminated with a second gate between them and the reticulocytes. Red blood cell inclusions usually have no effect on the reticulocyte enumeration, except for Howell-Jolly bodies. Thrombocytes pose no problem, except for occasional potential inclusion of giant platelets (Carter *et al*,1989;Chin-Yee *et al*,1991;Lee *et al*,1986).

1.2. The Importance of the Study

Clinicians rely heavily on marrow biopsy results to monitor and assess the success of bone marrow or peripheral blood stem cell transplants. An early indication of engraftment would allow for improved monitoring of patients. Clinicians are not only concerned with the regeneration of the transplant, but also need an early indication of rejection or graft failure, particularly in the cases of Servere Aplastic Anaemia (**SAA**). They do not always engraft following the first infusion. Additional information can be valuable in indicating what steps need to be taken to improve the situation earlier than would normally be undertaken.

Preconditioning prior to transplant can have an effect on the patient's bone marrow stroma. The Reticulocyte Maturation Index (**RMI**) response early in the transplant could be due to different and more intensive preconditioning regimes. It would therefor be beneficial to see what percentage of patients have early responses in relation to the preconditioning being used.

A measurement of cell harvest prior to infusion is an integral part of transplantation. We cannot, however, determine the quality of the harvest accurately by Mononuclear Count (**MNC**), Colony Forming Unit Granulocytic and Monocytic (**CFU-GM**) and Cluster designation **34+** (**CD34+**) counts alone. Failure of the **RMI** response early in the transplant can allow for the realization of inadequate cell harvest.

It would be strategically useful to eliminate the need for invasive and painful bone marrow biopsies usually performed on "day 14" post transplant. Particularly if cell

regeneration can be monitored accurately and reliably on peripheral blood in all three parameters Reticulocyte Maturation Index (**RMI**), Absolute Neutrophil Count (**ANC**) and Platelet Count (**PLT**).

1.3. The Problem Statement

The purpose of this study was to determine early response in Bone Marrow Transplant (**BMT**) and Peripheral Blood Stem Cell Transplant (**PBSCT**) patients at the Department of Haematology, Groote Schuur Hospital, by measuring the Reticulocyte Maturation Index (**RMI**) on a Coulter **EPICS Profile II** flow cytometer.

The study was divided into seven sub problems, so that we could evaluate all the aspects before coming to a final conclusion. The patients were first subdivided according to the various types of transplants they received, whether allogenic or autologous, whether **BMT** or **PBSCT** and including disease states.

Determination of a mean and median Response Time for **RMI**, **ANC** and **PLT** in each of the transplant groups was performed. A 95% Point of Response for each of the parameters was determined to establish a confidence limit. The information enabled us to establish the fact that the **RMI** does give an earlier indication of erythrocytic regeneration, as well as being a reliable engraftment indicator parallel to other leading transplant units.

Included in the study we aimed to sketch the effect of the preconditioning, richness of the **MNC**, total **CFU-GM** and **CD34+** on the early **RMI** response, by determining what percentage of the patients responded prior to the mean Response Time. We also aimed to prove in most cases the standard protocol, painful "day 14" bone marrow biopsy procedure, can be eliminated.

A Reference Range for the first forty days post transplant was calculated using **2SD.** This enabled us to establish that in a few cases peripheral blood counts post transplant created a prediction tool for a successful "12 month post transplant trilineage response".

This also allowed us to position out a pattern for expected recovery in patients throughout the first six months. Particularly when monitoring patients who have the potential for graft failure such as the Severe Aplastic Anaemia (SAA) transplants.

1.4. Delimitations

The study was limited to Bone Marrow and Peripheral Blood Stem Cell Transplant Patients at Groote Schuur Hospital. All transplant patients were monitored for a period of six months.

The study considered the following factors: -

- All transplant patients admitted to the Department of Haematology at Groote Schuur Hospital over the study period.
- The cost of monitoring the patients for the six month period.
- All transplant patients were monitored regardless of the presenting diagnosis.
- All transplant patients besides one were included in the study whether or not they achieved a morphological trilineage engraftment or not.
- There were four types of transplantation included in the study, allogenic and autologous stem cell harvests, and allogenic and autologous bone marrow harvests.
- Any patients receiving further harvests transplanted during the six-month period.
- Any patients receiving additional infusion boosters during the six month period.

The study did not consider the following factors:-

- Any supportive therapy the patient received during the six month period post transplant, i.e. blood products, vitamins and antibiotics. Mention of the possible effects of such therapies were however included in the discussion.
- Patients who received transplants outside the given dates of the study.
- Any patient that died within thirty days of transplantation.

1.5. Assumptions

- The first assumption was that all patients who underwent transplantation were successfully treated according to the department's protocols prior to transplant.

- The second assumption was that all patients did receive some sort of supportive therapy throughout the six-month study period.

- The third assumption was that all blood tested throughout the six month study period was correctly collected and was in fact the patient's own blood.

- The fourth assumption was that the flow cytometer was well maintained and produced accurate results.

- The fifth assumption was that all reagents and controls used throughout the study were of top production quality.

- The sixth assumption was that the secondary information used in the study i.e. absolute neutrophil count, platelet count, bone marrow results, **MNC**, **CFU-GM** counts, **CD34+** counts and preconditioning treatment, were all accurate and belonged to the patient concerned.

CHAPTER 2

Literature Review

2.1. Introduction

A graft consists not only of pluripotent stem cells but also contains pluripotent progenitors (**CFU_{GEMM}**), determinate progenitors (**CFU**c, **CFU**e and **BFU**e), precursors and mature red blood cells. In early regeneration following Bone Marrow Transplant (**BMT**) or Peripheral Blood Stem Cell Transplant (**PBSCT**), the progenitors take part in producing the mature blood cells after regeneration from the pluripotent stem cells. Reticulocytes mature to erythrocytes, and stay in the peripheral blood, they have a longer life span than the granulocytes (Arnold *et al*,1986). The Absolute Neutrophil Count (**ANC**), Absolute Monocyte Count (**AMC**) and the total White Blood Cell Count (**WBC**) are the standard means of determining bone marrow recovery following transplantation (Phillips *et al*,1990). Because reticulocytes are not influenced by infections, as are platelets and granulocytes, they could be considered the most stable and accurate means of determining erythrocytic and by inference granulocytic and platelet recovery. Flow cytometric methods (Davis *et al*,1989) allow for rapid and accurate counting of large numbers of reticulocytes, it also allows for the measurement of the amount of **RNA** within the cell, so that young and old reticulocytes can be separated (Dalal *et al*,1996).

2.2. Establishment of Automated Methods

The acceptance that the manual method of reticulocyte enumeration is inaccurate and limiting as far as only offering parameters such as percentage, absolute counts and corrected reticulocyte counts, has been a known fact for a number of years (Brecher *et al*,1950; Koepke *et al*,1986; Peebles *et al*,1981). The introduction of automated reticulocyte analysis opened doors to establishing superior counting methods, in such, allowing, not only for increased accuracy, but also for the creation of parameters that enable us to see earlier responses in erythrocyte production in numerous conditions including transplantation. There has been improved precision of reticulocyte counting by

flow cytometry; this encourages the use of the reticulocyte counts at not only low and normal ranges but high as well. Manual reticulocyte counts are not useful when the **CV** is greater than the counting range (Bajer *et al*,1994), Flow Cytometric Measurements (**FCM**) of reticulocytes however, allows for less statistical error because large numbers of cells are counted, as well as being faster and more reproducible (Lee *et al*,1986).

2.2.1. Thiozole Orange (TO) as a Dye

Dyes such as Pyronin Y, Acridine Orange, Propidium Iodide, Cyanine dye 3.3'-dimethyleoxacarbocyanide and Thioflavine T have been ruled out by various studies to be impractical in the use of **FCM** reticulocyte analysis (Jaccobberger *et al*,1984; Metzger *et al*,1987; Sage *et al*,1983; Tanke *et al*,1980 & 1983; Warren *et al*,1980).

With the introduction of Thiozole Orange (**TO**) as a reticulocyte dye in **FCM** (*Lee et al*,1986), it was found that ninety nine point nine percent of unstained erythrocytes could be gated to the left of a marker. When analysing a **TO** stained fluorescence histogram of a sample, mature red blood cells fall between the left and the middle marker and reticulocytes between the middle and the right marker. The data in the fluorescence histogram exclude white blood cells, which were outside the gates set in the dot plot (Figure 2 Page 32). **TO** allows for a higher saturation ratio than other dyes (Lee *et al*,1986). The use of **TO** in flow cytometric reticulocyte analysis in routine practice provides results with greater and higher precision than other fluorescent dyes (Van Hove *et al*,1990). The percentage of reticulocytes achieved, determined by **TO**, tend to be 15% higher than those determined by microscopy. (Serke *et al*,1993).

Studies over the years have proved **TO** to be an excellent dye choice for reticulocyte analysis by various methods of automation (Lee *et al*,1986; Davis *et al*,1989; Carter *et al*,1989; Ferguson *et al*,1990; Van Hove *et al*,1990). **TO** and automation have also been found to be more sensitive due to the increased fraction of mature reticulocytes counted, the largest source of error in manual counting is the difference between individuals on visual discrimination of the oldest reticulocytes (Kerry *et al*,1992)

2.2.2. The Co Efficient of Variance (CV)

Numerous studies were conducted to determine the correlation between automated reticulocyte analysis and the manual method, they all found the automated methods to be superior.

Carter *et al* (1989) demonstrated, flow cytometry precision counting by analysing specimens with reticulocyte counts between 1% and 11%. The specimens generated a mean **CV** of 3.1%, and a contrast with intra-observer variation and inter-observer variation for the manual counting of 11.9% and 20.8% respectively. Male and female counts when corrected to absolute values resulted in no sex difference. Similar results were obtained in other studies. Ferguson *et al* (1990) found that the reticulocyte count **CV** on the flow cytometer of 4.3% differed considerably to the manual method of 22.4%. Van Hove *et al* (1990) found that the average **CV** for a number of flow cytometric studies was 9.0% in samples with normal or high reticulocyte counts, improved **CV**'s were obtained than when percentages were low. They also discovered that when compared with the microscopic counts, the automated procedure produced the better **CV**. Davis *et al* (1990) found **FMC** reticulocyte counts to experience a **CV** of less than 5%, another study revealed that the manual reticulocyte counts produced a **CV** of >30% and **FCM** reticulocyte counts, a **CV** of <20% (Davis *et al*,1992).

2.2.3. Reproducibility and Stability

FCM reticulocyte counts were reproducible, in a study by Carter *et al* (1989), eight samples were evaluated at intervals of one hour. They were split into two batches and stored at 4°C and room temperature (**RT**) respectively. They were re-evaluated again at six and twenty four hours, resulting in excellent correlation between the two techniques for all of the seventy four specimens tested. Davis *et al* (1989) discovered no difference in reticulocyte percentage between fresh samples and samples stored at 4°C for 96 hours, or samples stored at **RT** for 48 hours.

2.2.4. Linearity

There was a discrepancy in analysis of linearity. Some studies found flow cytometric reticulocyte analysis linear at 0.5-5.8% (Van Hove *et al*,1990) and another only at a range of 1.8% to 30.1% (Ferguson *et al*,1990).

2.2.5. Incubation and Storage Time

In automated serial dilutions of a sample, there was no influence on the reticulocyte percentages. The counts remained unchanged during two to seven hour incubation times with **TO**. An incubation period exceeding seven hours artificially increased the counts, as well as those obtained after thirty hours storage. The incubation temperature was of less importance since there were consistent results over a wide temperature range (Van Hove *et al*,1990).

Cavill *et al* (1996) found a progressive fall in the automated reticulocyte count stained with **TO** over 192 hours, the results were similar regardless of whether the samples were stored at **RT** or 0-4°C. Although at **RT** a few samples with very high reticulocyte counts revealed a fall in the measured value over the first 24 hours, this was not the case at 4-8°C. The recommendation is that samples stored at **RT** should be analysed within 72 hours of being collected, and refrigerated samples may be safely analysed up to 120 hours after the samples have been collected.

2.2.6. Batch Precision

In 1990 Van Hove *et al* studied batch precision. He found that one sample produced thirty identical measurements, concluding that there was no instrument drift. Davis *et al* (1990) claimed that blood samples could be stored for 96 hours with no effect on results, batching of samples for that period of time reduced manpower needs.

2.2.7. Comparison of Automated and Manual Reticulocyte Counts

When comparing automated reticulocyte percentages and microscopic counts, somewhat higher values for the flow cytometer were to be expected. This was due to higher sensitivity of the flow cytometric technique to detect reticulocytes with minor amounts of **RNA** (Lee *et al*,1986;Kraaijenhagen *et al*,1996). There was weaker correlation between

flow cytometer and microscopic values probably resulting from the manual gate settings for the reticulocytes (Van Hove *et al*,1990). We need to be aware of artifacts such as **HJ** bodies as these samples still require microscopic viewing (Davis *et al*,1989; Van Hove *et al*,1990; Lofsness *et al*,1994).

2.2.8. Instrument Precision

Studies conducted by single-laboratory **FCM** reticulocyte counting revealed precision differences with regard to the fluorochrome, stain, instrumentation and data analysis software algorithms used to distinguish reticulocytes. Therefore, results from one single institution cannot not be regarded as inter-laboratory precision of **FCM** reticulocyte counting (Davis *et al*,1992).

Ultimately there was good correlation between manual counts and instrument counts, provided that analysis was performed within two days after blood donation, longer storage should be avoided, since increase in reticulocyte percentages was usually seen, followed by a decline (Lee *et al*,1986; Von Hove *et al*,1990).

Davis *et al* (1992) designed a study, primarily to gather data on inter-laboratory precision and correlation of reticulocyte counting by different **FCM** methods. A second goal was to validate **RMI** measurements, define the proportion of highly fluorescent reticulocytes, and to determine the inter-laboratory precision of this new parameter. Three hundred and ten samples were evaluated on eleven different instruments. Correlations between sites did not differ significantly. The ability of each laboratory to distinguish normal from abnormal results depended on a carefully defined normal range, which was method dependent. Their results reinforce the view that each laboratorv performing **FCM** reticulocyte analysis establishes a normal range of their own, with any significant methodological precisions needed as there are key factors influencing recognition of abnormality.

2.3. Indicators of Aplasia

When using cytotoxic drugs or radiotherapy it is important to establish at what point, bone marrow aplasia occurs. d'Onofrio *et al* (1996) reported that bone marrow aplasia is

calculated as: less than 0.1 $x10^9/l$ for **WBC** and **ANC**, less than 30 $x10^9/l$ for un-transfused **PLT**, less than 10 $x10^9/l$ for **RET**, and equal or less than 1% **HFR**. Putative variables were as follows; **WBC** above 1 $x10^9/l$, **PLT** above 30.40 or 50 $x10^9/l$ without transfusion, **RET** above 15.20 or 30 $x10^9/l$, **HFR** above 1, 2, 3, 4 and 5% and the sum of **HFR** and **MFR** above 5 or 10%.

2.4. Criteria of Engraftment

In all cases severe pancytopaenia was observed after a conditioning regime. Therefore there was a need for early detection of bone marrow recovery (Davis *et al*,1989; d'Onifrio *et al*,1996). Over the years various methods of erythroid engraftment have been evaluated for different types of transplantation. Common ground was established when evaluating bone marrow engraftment.

Post transplant granulocytic recovery criteria included an **ANC** greater and equal to 0.50 $x10^9/l$ (Davis *et al*,1989; Chin-yee *et al*,1991;Greinix *et al*,1994; Davis *et al*, 1992; d'Onofrio *et al*,1996), a **WBC** of greater than $1.0x10^9/l$ (Greinix *et al*,1994). Erythroid engraftment was indicated by various methods, reticulocyte percentage greater than 1.0% (Davis *et al*,1989), Absolute Reticulocyte Count greater or equal to 0.50 $x10^9/l$ (Davis *et al*,1989), an increase of **MFI** above 77 (Davis *et al*,1989), an **HFR** count above 0.5 $x10^9/l$ or a **HFR** percentage greater than 5% (Greinix *et al*,1994; d'Onifrio *et al*,1996) and a **RET** of greater than $20x10^9/l$ (Greinix *et al*,1994; d'Onifrio *et al*,1996). Graft failure is defined by marrow aplasia after twenty eight days (Davis *et al*, 1992; Greinix *et al*,1994).

2.5. Indicators of Engraftment

2.5.1. Reticulocyte Count as an Indicator of Engraftment

When investigating the sensitivity of the flow cytometric reticulocyte counts against manual methods, bone marrow regeneration following transplantation became a point of interest (Davis *et al*,1989). It was found that the automated methods generated fewer fluctuations and detected reticulocyte reappearance one to two days earlier than the manual method (Van Hove *et al*,1990). Initial reticulocyte frequencies were found to be normal, a decrease was seen following cytostatic therapy which returned to normal

following bone marrow recovery (Davis *et al*,1992). In another study reticulocyte counts showed recovery two to three days prior to the neutrophil or platelet counts and detect the recovery of marrow function earlier in approximately 50% of the patients, suggesting that the reticulocyte count was the best early predictor (Lazarus *et al*,1991).

It was established that reticulocyte counts on a whole responded earlier than either neutrophil or platelet counts alone (Davis *et al*,1989&1990; Van Hove *et al*,1990; Chin-Yee *et al*,1991; Greinix *et al*,1994). There were however, complications such as oscillations in the reticulocyte count, which could confuse the prediction of bone marrow recovery, especially during periods of pancytopenia. This was possibly due to transfused reticulocytes, because banked blood has normal numbers of reticulocytes. The platelet count is the least reliable method, because of the need for platelet support particularly in the early stages post transplantation. The neutrophil count is usually the most reliable because of the magnitude of response from transplantation until recovery, it is considerably greater than any other cell type because they are unaffected by fluctuations in frequency, but can be affected by sub clinical infections (Lazarus *et al*,1991).

Lazarus *et al* (1991) demonstrated that some transplants show that recovery can not be identified earlier by using populations of younger reticulocytes, but the magnitude of increase from the lowest count to a second successive increase was significantly greater using the younger reticulocyte populations. They also noted that if tests for bone marrow recovery were obtained daily rather than three to four times a week, data would reveal less random variation. Therefore obtaining daily reticulocyte counts and subdivision of the reticulocyte **RNA** distribution (to identify younger cells) could improve monitoring of bone marrow transplant patients by reticulocyte counting. Because flow cytometric analysis of reticulocytes is more sensitive and precise Lazarus found that their method counted several logs, and more cells than any other method. Therefor their manner of analysis could predict recovery earlier than by blood smear in eighty percent of the patients studied. Although transfused reticulocytes can interfere with analysis, this may be overcome by quantitatively assessing reticulocyte age. Lazarus felt that it was important to have the means to demonstrate recovery of individual blood cell types after

marrow transplantation. This was because no significant differences were noted in determining bone marrow recovery using combined reticulocyte, neutrophil and platelet data for type of marrow transplant (autologous or allogeneic), sex or tumour type. In addition there was no difference between allogeneic and autologous patients measured by platelet or reticulocyte counts. Analysis revealed that the largest difference between reticulocyte and neutrophil counts predicting early engraftment was observed, particularly in allogeneic marrow transplant patients when compared to autologous marrow transplants. Reticulocyte recovery was predicted earlier by flow cytometric analysis in comparison to blood smear analysis in autologous transplant patients but not in allogeneic transplant patients. Flow cytometric reticulocyte counts were also not significantly different to platelet counts in allogeneic transplant patients.

Studies became more specific as individual researchers began to evaluate the various means of detecting early reticulocytes, and correlating them to other transplantation recovery indicators.

2.5.2. Mean Fluorescent Index (MFI) as an Indicator of Engraftment

The Mean Fluorescent Index (**MFI**) is the channel in which the most reticulocytes fall into in relation to the fluorescent dye used. Reticulocytes exhibit dim, diffuse cytoplasmic fluorescence, so samples of low reticulocyte percentage may have either a low or normal **MFI** value. Low reticulocyte percentages and a low **MFI** are indicative of a true hypoproliferative erythropoietic state. Patient samples with low or normal reticulocyte percentage showed a wide range of **MFI** values, indicating that the **MFI** parameter provides additional information regarding the erythropoietic response (Davis *et al*,1989).

Davis *et al* (1989) conducted two studies on auto**BMT**. In both, the **MFI** increase correlated with the successful return of bone marrow function and paralleled a rise of **ANC**. In the first study ninety two percent of the patients with successful bone marrow engraftments had a recovery period of ten to thirty four days, sixty four percent of the patients showed a rise in **MFI** value prior to **ANC**. Thirty six percent showed an **ANC**

response prior to or on the same day as the increase in **MFI**. The reticulocyte percentage did not rise above 1% until one to seven weeks after the increase in neutrophil counts.

In a second study by Davis *et al* (1989) also involving auto**BMT**, a rapid engraftment was seen in eighty percent of the patients within ten to twenty days after transplantation. Delayed engraftment was seen in fifteen percent of them, and there was no evidence of engraftment in five percent of the patients. The **MFI** was the most sensitive indicator of engraftment. At no time did the count decline below the defined threshold for their requirement of graft achievement (77). The **MFI** rise preceded a rise in **ANC** in sixty three percent, and on the same day in sixteen percent of the patients. In the remaining fifteen percent the rise in **ANC** preceded the **MFI** by only four to six days. The average time of engraftment was fifteen days for the **MFI**, eighteen days for the **ANC**, forty days for the reticulocyte percentage and forty nine days for the absolute reticulocyte count. The **MFI** and **ANC** values indicated engraftment in less than half the time from transplantation compared to the other traditional reticulocyte parameters.

In 1990 Davis *et al* pointed out that the **MFI** could identify three patterns of marrow engraftment (early, delayed, failed), while the standard reticulocyte percentage and absolute count showed no significant changes or fluctuations attributable to red cell transfusions. Because **TO** also stains **DNA** and can allow for interference, their suggestion was to exclude the counts in the highest fluorescent channel of the histogram from the data analysis. Nucleated cells are then not falsely included in the reticulocyte enumeration.

In 1991 Chin-yee *et al.* monitored auto**BMT** patients after ablative chemotherapy, using the **MFI** as the indicator for engraftment compared to the **ANC**. A forty percent earlier increase in **MFI** was seen. This preceded the increase in the absolute reticulocyte count.

2.5.3. High Fluorescent Reticulocyte (HFR) as an Indicator of Engraftment

The **HFR** is the region where the most fluorescence is exhibited, and is indicative of the most immature reticulocytes as they contain the most **RNA**. Use of this parameter is

most beneficial, particularly following bone marrow ablation and pending recovery, because the moment early eryhtroid cells are released into the blood, they would be detected, even when the reticulocyte percentage is still low.

In 1992 Davis *et al* evaluated the total reticulocyte count and the **HFR** response in twenty two auto**BMT** and fourteen allo**BMT** patients. A median **HFR** was seen within fourteen days post auto**BMT** and twelve day post allo**BMT**. The **HFR** rise preceded the onset of a significant rise in reticulocytes in fifty nine percent of the auto**BMT** patients and sixty four percent of the allo**BMT** patients. **HFR** recovery was seen in one auto**BMT** and eight allo**BMT** patients prior to the **ANC**, and in two auto**BMT** and three allo**BMT** patients simultaneously. In nine auto**BMT** and three allo**BMT** where **HFR** rise followed **ANC** recovery, the **HFR** was not significant. **HFR** although sensitive in detecting the onset of erythropoiesis, was less specific in indicating prognosis.

In 1994 Greinix *et al* evaluated **HFR** and **ANC** in twenty seven allo**BMT** and twenty one auto**BMT**. The **HFR** response was seen within seven days in allo**BMT** and twelve days in auto**BMT**. The **HFR** response was also seen seven and two days earlier than the **ANC** response.

In 1996 d'Onofrio *et al* evaluated nineteen allo**BMT**, twelve auto**BMT** and twelve auto**PBSCT**. Total **WBC**, **ANC**, **RET** and sub-fractions were measured. Reticulocytes were subdivided into three subgroups **LFR**, **MFR** and **HFR**. Significant median recovery times for **HFR** were eleven days for allo**BMT**, fourteen days for auto**BMT** and eight days for auto**PBSCT**. **ANC** recovery was seen within twenty days for allo**BMT**, twenty four days for auto**BMT** and fourteen days for auto**PBSCT**. A greater than $30x10^9/l$ **PLT** recovery at twenty one days for allo**BMT**, twenty four days for allo**BMT** and nine days for auo**PBSCT** was found.

In 1997 Testa *et al* evaluated five auto**BMT** and thirty one auto**PBSCT**. Reticulocyte recovery in auto**PBSCT** ranged from day two to day thirty. The **HFR** peak percentage occurred in auto**PBSCT** at day eight and in the auto**BMT** at day twenty four. The raise

in **HFR** preceded the total reticulocyte count increase by six days in the auto**PBSCT** and sixteen days in the auto**BMT** patients. Finally, the rise of both **HFR** and total reticulocyte count preceded the **Hb** recovery.

In 2000 George *et al* described a **HFR** reading of 2% as a recovery indicator in auto**PBSCT,** this occurred at a mean and median of eight days following transplantation in lymphoma patients. The **HFR** preceded the **ANC** in twenty four of the twenty five cases by three days. They also found a ninety six percent engraftment with an early **HFR.**

2.5.4. High Fluorescent Rate plus Medium Fluorescent Rate (HFR+MFR) as an Indicator of Engraftment

Another method of early reticulocyte response is adding the number of high fluorescing reticulocytes to the normal fluorescing reticulocytes (**HFR+MFR**).

In 1994 Batjer *et al* evaluated thirty two allo**BMT** and eight auto**BMT** using the (**MFR+HFR**) as an indicator for erythrocyte recovery. The **MFR+HFR** mean response was seen within thirteen days for both, fifteen days for the **WBC** count for both; sixteen days for the **RET%** and seventeen days for the **VRET%** for both the allo**BMT** and the auto**BMT**.

In 1997 Remacha *et al* published one of the first reported studies using allo**PBSCT** recipients. It demonstrated that the **MFR+HFR** was the first parameter to recover after both allo**PBSCT** and auto**PBSCT**.

In 2001 Torres *et al* examined thirty allo**PBSCT** and thirty auto**PBSCT**, using the **MFR+HFR** which they termed the **IRF** (Immature Reticulocyte Fraction). They found a response on day nine in the autologous transplants and a thirteen day response in the allogeneic transplants, both responding earlier than the **ANC**.

2.5.5. Reticulocyte Maturation Index (RMI) as an Indicator of Engraftment

With the introduction of automated reticulocyte counts, parameters other than the reticulocyte percentage, absolute reticulocyte count and corrected reticulocyte count were created. Grading of the fluorescence exhibited by **TO,** was used to divide the cells into "mature" Low Fluorescing Reticulocytes (**LFR**), "normal" Medium Fluorescing Reticulocytes (**MFR**) and "immature" High Fluorescing Reticulocyte (**HFR**). **HFR** is the indicator for the early or immature reticulocytes and is one of the parameters used extensively on its own or in combination with other parameters to detect early eryhtrocytic response in the peripheral blood (Tarallo *et al*,1994;Tichelli *et al*,1990).

Some studies used the **MFR** as an erythroid recovery indicator. This approach, though useful within a single laboratory using a single protocol and instrument, presented problems in the comparison of different models of **FCM**. Various **FCM** instrument models use different methods of fluorescence capture and logarithmic amplification of light-generated electronic signals, such as the numeric values of fluorescence intensity, and hence **MFI** units differ. An approach to standardise **TO** stained reticulocyte quantification by multipurpose **FCM** instrument use, is to incorporate the expression of Reticulocyte Maturation Index (**RMI**) as the fraction of highly fluorescent, more immature reticulocytes. The **RMI** is calculated by determining the fraction of reticulocytes in the **HFR** relative to the total reticulocyte population (**RMI** = number of high fluorescent (immature) reticulocytes/total number of reticulocytes). Work with **TO** has indicated that the normal range for **RMI** values by this method was 0.2-0.5 IU (Bauer *et al*,1993; Davis *et al*,1992).

In 1996 Dahal *et al* evaluated twenty nine allo**BMT** and eight auto**BMT** using the **ANC** and **RMI** for transplant recovery. All thirty seven patients engrafted, with a median of seventeen days for the **RMI** and nineteen days for the **ANC**. In twenty two patients the **RMI** signalled first, in thirteen the **ANC** signalled first and in two they occured on the same day. He felt that the time difference between the **RMI** and **ANC** signals were independent of the underlying disease or the type of **BMT**. The rising trend of the **RMI** was persistent in thirty six of the thirty seven patients, enabling a confident prediction of

engraftment, whereas the **ANC** fell below the threshold in six patients for up to six days after signalling engraftment.

2.5.6. Discussion on Indicators of Engraftment

According to all the studies conducted involving early eryhtrocytic response post **BMT** and **PBSCT**, the erthrocytic response using parameters of the reticulocyte count were the first to respond in the majority of the cases (Dahal *et al*,1996; Davis *et al*,1989). In a few cases there was a combination response of the reticulocyte parameter and the granulocyte count (Dahal *et al*,1996;Davis *et al*,1989) and in minimal cases the granulocyte response was seen prior to the reticulocyte parameter response (Dahal *et al*,1996;Davis *et al*,1989).

Davis *et al* (1992) found an immediate drop in **HFR** during pre-transplant conditioning while the total reticulocyte numbers fell more slowly. Even when **HFR** reached zero, reticulocytes were always detectable. d' Onofrio *et al* (1996) found that suppression of the erythropoietic activity is usually demonstrated by the fall of **RET** below 10 $x10^9/l$. A zero value for the reticulocyte count was never seen, even if no reticulocytes at all could be seen on the peripheral blood smear during the period of aplasia. The impossibility of obtaining zero reticulocytes with the instrument probably results from autofluorescence of mature reticulocytes.

d'Onofrio *et al* (1996) also found that the aplastic phase appeared to be more severe in auto**BMT** than in allo**BMT,** and more prolonged both in auto**BMT** and allo**BMT** compared to the auto**PBSCT**. The patients in the auto**BMT** group showed the longest period of granulopoietic and thrombocytic suppression, and in particular most patients did not recover a stable un-transfused **PLT** count for at least a month. Patients with **PBSCT** on the other hand usually experienced a very short period of thrombocytopaenia. The **WBC** usually remained below 0.1 $x10^9/l$ for shorter times than the **ANC** due to the very rapid increase in the monocyte counts, which contributed to the early rise in total **WBC**. According to Grienix *et al* (1994) severe reticulocytopaenia was the earliest, consistent and most prolonged phenomenon in the auto**BMT**. However, d'Onifrio *et al*

(1996) and Testa *et al* (1997) found earlier recoveries in both auto**BMT** and auto**PBSCT**, indicating a "pattern of recognition" seen in autologous transplants.

Erythropoiesis is linked to iron metabolism in that it is largely and continuously dependent on a high supply of circulation iron. Both Greinix *et al* (1994) and Testa *et al* (1996) found that recovery of erythropoiesis occurred earlier and more rapidly in patients receiving erythropoietin after marrow infusion. In addition, erythropoietin did not impair myeloid regeneration. They regarded treatment with **G-CSF** a stimulant for granulocytic recovery only, and this did not effect erythroid regeneration since a rise in **HFR** and **ANC** are seen at almost the same time. But Kuse *et al* (1996) felt that because higher median results in **HFR** and **MFR** were seen following therapy, **G-CSF** was not lineage specific and may also stimulate the eryhtroid precursors. Allo**BMT** patients receiving erythropoietin had significantly shorter response times than the **ANC**, where the **ANC** response was similar to those not treated with erythropoietin. In patients receiving **G-CSF** after marrow infusion both **HFR** and **ANC** responded at almost exactly the same time. In 1997 Testa *et al* found that in patients without any cytokine treatment **HFR** recovery was significantly earlier than the **ANC**. An absence in a fall of plasma ferritin level after **SCT** suggests that the mobilization of iron stores is not the main mechanism for obtaining additional iron for erythropoietic recovery.

Davis *et al* (1992) felt although resumption of granulopoiesis and erythropoiesis appear to occur in parallel, variations are seen in the relative timing of the appearance of mature granulocytes and reticulocytes. Relationships of change between the reticulocyte levels and neutrophil counts vary between patients, but usually there is a general consistent rise in reticulocyte counts. Substantial delays in onset of erythropoiesis or granulopoiesis can be noted in a small minority of cases. Slow onset of erythropoiesis can be seen in **ABO** mismatches.

Automated reticulocyte counts are by far the most reliable indicator of engraftment. Reticulocytopaenia is preceded by total disappearance of the **HFR** fraction; and is the earliest sign of haemopoietic function after conditioning treatment. The time between the

automated reticulocyte count response and the total reticulocyte count response is different (Davis *et al*,1989; Batjer *et al*,1994). In context, the relatively long delay observed in the auto**BMT** patients between the **HFR** response and that of the total reticulocytes may reflect an initial wave of erythropoietic rescue sustained by late erythroid progenitors. This was followed later by more constant erythroid differentiation involving early and late erythroid progenitors (Davis *et al*,1989). **HFR** although sensitive to the onset of erythropoiesis, is less useful in indicating and predicting erythropoietic rescue. Erythropoiesis is strictly linked to iron metabolism in that it is largely and continuously dependent upon a high supply of circulating iron.

In conclusion, the reticulocyte parameters regardless of whether it is the **MFI**, **HFR**, **MFR+HFR** or the **RMI**, are the best indicators for early erythrocytic response in **BMT** and **PBSCT**. Davis *et al* (1993) was partial to the **HFR** method of **RMI** expression and claimed that it provided a superior means of inter-laboratory standardisation and clinical comprehension of this useful diagnostic parameter in the clinical haematology laboratory. Pappas *et al* (1992) stated that the **RMI** can provide an independent measurement of reticulocyte **RNA** content and erythropoietic activity, and may be useful in predicting **BM** engraftment or further subclassifications in anaemias. In 1996 Dahal *et al* explained that the **RMI** does not correlate with the Reticulocyte Count (**RC**) either by **FACS** or manual methods, but the combination of **RMI** and **ANC** predicted earlier engraftment than the **RMI** or **ANC** separately. This factor could help to overcome the uncertainty caused by **ANC** fluctuations due to laboratory error. Moreover, the bilineage engraftment signal of the **RMI/ANC** combination could be more reliable in patients who have received growth factors. A potential limitation of **RMI** in erythroid regeneration is seen in some **ABO**-incompatible **BMT.** However, using the **RMI/ANC** combination criteria would eliminate this problem.

Remacha (1997) believed that the same kinetics of haemopoietic reconstitution seem to operate in both **PBSCT** and **BMT**. The main advantages of **PBSCT** over **BMT** are the faster neutrophil and platelet recoveries. The recovery of absolute **RET** and **MFR+HFR**

were also faster in **PBSCT** than in **BMT** and the pattern of these recoveries held true in both.

2.6. Haematopoietic Progenitors

Stem cells can self-renew and differentiate into haemopoietic progenitor cells to sustain haemopoiesis. The progenitors are multipotent (**CFU**-granulocyte-erythrocyte-macrophage-megakaryocyte) or committed to erythroid series (burst-forming unit-erythrocyte and **CFU-E**), the granulo-monocytic lineage (**CFU**-granulocyte-macrophage) and the megakaryocyte **CFU** series. The progenitors in turn differentiate into morphologically recognisable precursors that mature into terminal elements circulating in the peripheral blood (Testa *et al*,1997).

Even though blood cells ultimately arise from a totipotential haemopoietic stem cell, early regeneration of cell populations after transplantation may be from committed progenitor cells. Because the platelet distribution is skewed it is possible that the distribution of platelets is unimodal, whereas the reticulocyte and neutrophil distributions show evidence of bimodality. The rate of recovery of the three formed elements, could be independent of each other (Lazarus *et al*, 1991).

In 1989 Arnold *et al* studied forty one **BMT** using reticulocyte percentage, **ANC** and **PLT** as indicators of engraftment. Bone marrow histology was evaluated at "day 7" and "day 42" and a correlation was drawn with the haemopoietic progenitors infused (**CFU**c, **CFU**e and **BFU**e). The reticulocytes were the first to recover with a median time of twenty two days (range fourteen to fifty days), followed by the **ANC** recovery in a median time of twenty four days (range fourteen to fifty two days). Finally, the **PLT** recovery time was twenty one days (range thirteen to seventy days). Bone marrow histology revealed pure erythroid or granulocyte colonies at "day 7" and normal morphology at "day 42". No correlation between the reticulocyte count and the haemoglobin concentration was found. All patients had a moderate anaemia during the first two months after **BMT**. The correlation between the number of transplanted nucleated cells and the reticulocyte percentage and the **ANC** was significant.

There is considerable disagreement regarding predictive value of the nucleated cell counts in transplant patients. Only Niederwiser *et al* (1983) found a significant correlation between the number of nucleated cells transplanted and the granulocyte recovery. This is in contrast with Faille *et al* (1981), Jansen *et al* (1983), Harada *et al* (1983) and Torres *et al* (1985). Discrepancies in results could be due to small numbers of patients studied. Other problems are, the range of nucleated cells transplanted was too narrow for testing a correlation with the time to granulocyte recovery, which depends on more factors than the reticulocyte recovery. Henon *et al* 1998 found that **MNC, CFU-GM, CD34+** and **CD38+** correlated inconsistently with haematopoietic recovery parameters. They suggested that the **CD34+** and **CD38+** subpopulations be composed of committed progenitor cells involved in early trilineage engraftment.

Arnold *et al* (1989) found the determination of the colony forming unit of granulopoiesis (**CFUc**) was their best parameter, since the numbers of erythroid progenitors showed large variations, even in the normal controls. In the auto**BMT,** haemopoietic progenitors were drastically reduced or completely absent, but haemopoietic reconstruction occurred in all their patients. In allo**BMT**, significant correlation's between the number of **CFUc** transplanted and the time to granulocyte recovery were found, although others found no correlation between the number of **CFUc** transplanted and the time of granulocyte recovery.

Observations showed that a transplanted nucleated dose of less than 1 $x10^8$/kg b.w. prolongs the time to recovery. This is especially true of platelet recovery. The same was found to be true for **CFUc** dose of less than 1 $x10^8$/kg b.w. In accordance with the literature, detectable progenitor incidence occurred only in the second week after **BMT**. For early evaluation of the harvest, the bone marrow cellularity, measured as nucleated cell count, is a simple and immediately available parameter. This is in addition to bone marrow morphology and careful evaluation of granulocyte and reticulocyte recovery of the peripheral blood.

In 2001 Torres *et al* found that **CD34+** infused cells showed a statistical influence on erythroid engraftment in autologous transplantation. Mougi *et al* (1997) determined the optimal time for Peripheral Blood Stem Cell Harvest (**PBSCH**) recovery was not only reliant on **CD34+** counts but immature leucocyte information and **HFR** should also be considered.

2.7. Graft Failures

Graft failure is possibly the clinicians biggest fear. Therefore, evaluating reticulocyte recovery is not only valuable in determining transplant success, but is a tool that would be of great importance in detecting early graft failure. Over the years, there have been numerous publications on determination criteria for a failed engraftment.

In 1976 Lohmann *et al* found that patients with reticulocyte counts less than 10 000/ml were at extremely high risk of succumbing to marrow aplasia. There were no survivors thirty six months post diagnosis. Seventy five percent with reticulocyte counts greater than 10 000/ml survived three years. This allowed Bunjies *et al* in 1990 to classify "early graft failure" as no reticulocyte response prior to "day 50", and "late graft failure" as no response after "day 50". They managed to reverse "graft failure" in some cases where there were residual reticulocytes, but failure was seen in cases where there were no residual reticulocytes. Van den Berg *et al* (1990) did a comparison of biopsy findings with clinical and laboratory data. There was a correlation between the amount of erythroid cells and the day of appearance of reticulocytes. Absence of clustering of the haematopoietic cells in four of the five patients was associated with either failure of engraftment or early leukaemic relapse. Variables such as infections and administration of possibly myelosuppressive drugs did not influence the bone marrow findings.

Lazarus *et al* 1991 in their study found that the reticulocyte count was the first to recover post transplant. This reticulocyte recovery did not differ in any of their patients, with the exception of one, in whom the bone marrow did not recover before death. In 1992 Davis *et al* found a median reticulocyte count of 23.6 $x10^9/l$ at "day 21" in the successful engraftments, and 6.6 $x10^9/l$ in the inadequate engraftments. Engraftment ultimately

occurred in all cases with reticulocyte counts greater than 15 $x10^9$/l. This occurred within three weeks following transplantation. Thirty six percent of the autoBMT and fourteen percent of the alloBMT had inadequate engraftment. However two of these cases relapsed at two and four months post BMT and would not have been considered to have had primary engraftment failure.

In 1996 Gerritsen *et al* examined "graft failures" in fifty five children who were non-genotypically matched and T cell depleted. The aim was to investigate simple haematological laboratory parameters to predict engraftment or graft failure. None of the children needed myeloablative-conditioning as they had SAA and poor risk leukaemias. They found normal recovery of granulocytes and the reticulocytes in the PB, as well as the presence of trilineage haematopoiesis in BM biopsies. This was seen between twelve and twenty days post BMT. In the first two months post BMT the number of reticulocytes/1000 RBC was significantly higher in patients grafted for SAA than patients grafted for leukaemia. Reticulocyte appearance was significantly delayed in. patients with isohaemagglutinins against the donor. "Graft failure" was defined in ten cases by the disappearance of reticulocytes after transplant. This conclusion was made, as the reticulocyte count was the most accurate. In five cases, the reticulocytes remained present in the PB post transplant.

2.8. Early Bone Marrow Biopsies

Because the MFR+HFR indicated regeneration a day and a half earlier than the WBC count and four days earlier than the RET%, Batjer *et al* (1994) felt that the combination of these three parameters for marrow recovery could decrease the number of marrow aspirations performed to monitor marrow engraftment. Bone marrow aspiration with its pain and cost might be necessary only when the peripheral blood parameters deviate significantly from the predicted course. The recommendation for the measurement of reticulocyte maturity, as early indication of bone marrow regeneration during transplantation engraftment, is a consideration. In general, the decrease in frequencies of all cell types reflected the cytotoxic treatment that the patient received during the first two weeks of evaluation (Lazarus *et al*,1991).

2.9. Long Term Response

Long term recovery is another aspect of transplantation; there is always a need to apply all means available to establish a positive prognosis, although at this stage there is no prediction tool or set of criteria that would guarantee that. We can at this stage only monitor long term effects.

In 1989 Arnold *et al* found that long term studies of the erythrocytes revealed a macrocytosis and slight hypochromia. Although bone marrow morphology showed no evidence of recurrent disease, all patients had subnormal bone marrow cellularity and subnormal numbers of progenitors up to three years post transplantation. Pluripotent stem cells of donor origin repopulate the marrow of the supralethally conditioned host after bone marrow transplantation. The decreased bone marrow cellularity despite normal production of peripheral blood cells can be explained by an expansion of the haemopoietic tissue to atypical medullary and extramedullary sites.

CHAPTER 3

Materials and Method

3.1. The Flow Cytometer

3.1.1. Principle

The flow cytometer measures the fluorescence and laser light scattered in the forward and right angle directions by microscopic particles passing through the laser beam. Forward light scatter correlates particle size, and side scatter correlates to the granularity or complexity of the cell. The intensity of fluorescence gives the affinity of the sample for certain dyes, or the inherent fluorescence of the sample.

A particulate sample for the system must be suspended in a liquid. The system measures up to 10 000 particles per second. For light scatter measurements, particle diameter can range from 0.5 to 40 micrometers. For fluorescence measurements the sample can be molecular or as large as 40 micrometers in diameter.

For better particle discrimination, the system correlates and stores on a "particle-b-particle" basis several measures of particle characteristics and graphically displays the distribution of those characteristics within the population.

3.1.2. Operation

Samples of analysis are presented to the system in a small test tube, where they are delivered by syringe. A probe draws up a preset volume of sample, and the syringe delivers the sample to the flow cell at a preset rate. Sample flow is guided into the sheath stream at the flow cell with sheath from a container, which is automatically controlled from the computer. A "bioSense" flow cell (250 micromillimeter sense-in-quartz) introduces the sample into the sheath stream to create a flow of sample particles in single file. The beam-shaping lens assembly focuses the laser light, and the sample is

illuminated as it passes through the laser beam. After illumination, sample and sheath are collected in the waste bottle.

Laser stands for Light Amplification by Stimulated Emission of Radiation. When an atom absorbs energy, it is pushed into a higher energy state; its electrons are put into higher orbitals. To go to a more energetically favoured state the electrons drop to lower orbitals. The energy differences between lower and higher orbitals produce a photon light. This is called spontaneous emission. Since there are only a few different orbital transitions for a particular substance, only a few different wavelengths of light are emitted.

The air-cooled argon laser illuminates cells passing through the sensing area and measures their fluorescence and light scatter; a unique light source is thus needed. The direction, wavelength and intensity of the light must be as constant as possible. Only a laser produces light of this quality.

The argon laser uses electronic electrical energy to excite the argon ions into a higher state. Mirrors at either end of the plasma tube reflect the light from spontaneous emission back and forth down the middle of the plasma tube. When a photon of light passes near an excited argon ion, the ion emits a photon of light at the exact same wavelength, polarization and direction as the incident photon. This is called stimulated emission. The beam from the laser is about 650 micrometers in diameter and focused to increase the intensity and to keep from illuminating more than one cell at a time, and making it as uniform as possible. The beam first passes through cylindrical lenses, which reduce width and height, producing an elliptical beam.

After beam shaping the laser beam passes through the sensing area of the flow cell tip. The sensing area is a long hollow chamber. Cells pass through the middle of the laser beam in this chamber. As the cells pass through the middle of the laser beam, they scatter the laser light and emit fluorescent light from fluorescent dyes attached to the cells.

A built in microscope for viewing the sensing area detects the light information and a forward scatter detector measures particle size sensitivity. The bioSense flow cell is a mirror for increased sensitivity. Fluorescence lenses detect light which passes through a first lens which directs light to the side scatter Photo Multiplier Tube (**PMT**), a second lens sends light in the opposite direction through a pin hole which is picked up by a third lens which recollimates light and sends it to a **PMT**. In the **PMT** there are filter slots which allow for the insertion of filter and beam splitter cartridges for measuring different colours of fluorescence. The sensor computer controls the laser power, sample delivery system, and the voltage supplied to each **PMT**. The sensor computer receives instructions from tests defined at the data acquisition computer or the software. Ultimately the **PMT** multiplies the signals, which are then processed by an Analog-to-Digital Converter, which integrates and amplifies either logarithmically or linearly signals received. The peak height of the integral signal is proportional to the total amount of fluorescence from a cell while the peak signal is proportional to the maximum concentration of fluorescence within the beam at one time. The signals are amplified, compensated for overlap in fluorescence, where the information is digitized and placed within a histogram for personal analysis by the operator (Figure 1 Page 30) (Coulter Operators Manual for the **EPICS Profile II** flow cytometer,1993).

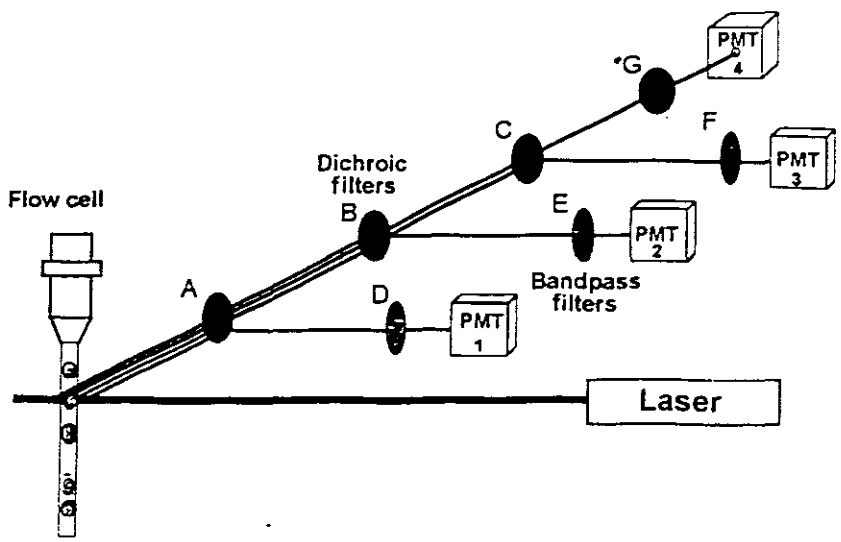


Figure 1: Internal view of a flow cytometer, an argon-ion laser giving blue light (488nm), the detectors (**PMT**) collects the light and measures four different colours, dichromic filters (picture from: Flow Cytometry second edition M.G. Omerod (1999)).

3.2. Instrumentation

The instrument used in the study was the Coulter **EPICS Profile II** flow cytometer. It was equipped with an air cooled argon laser set at a 15 mw output and a 488 nm laser line, and a detector compartment which contained two fluorescent detectors, a side detector, two filter slots and collection filters. The fluorescence was measured with 525-nm band pass interference filters and collected on three generation log amplifiers.

The Coulter **EPICS Profile II** flow cytometer was the routine instrument in the department at the time of commencement of the study, and it was also the instrument with which we established the normal range for our population group, at Groote Schuur Hospital.

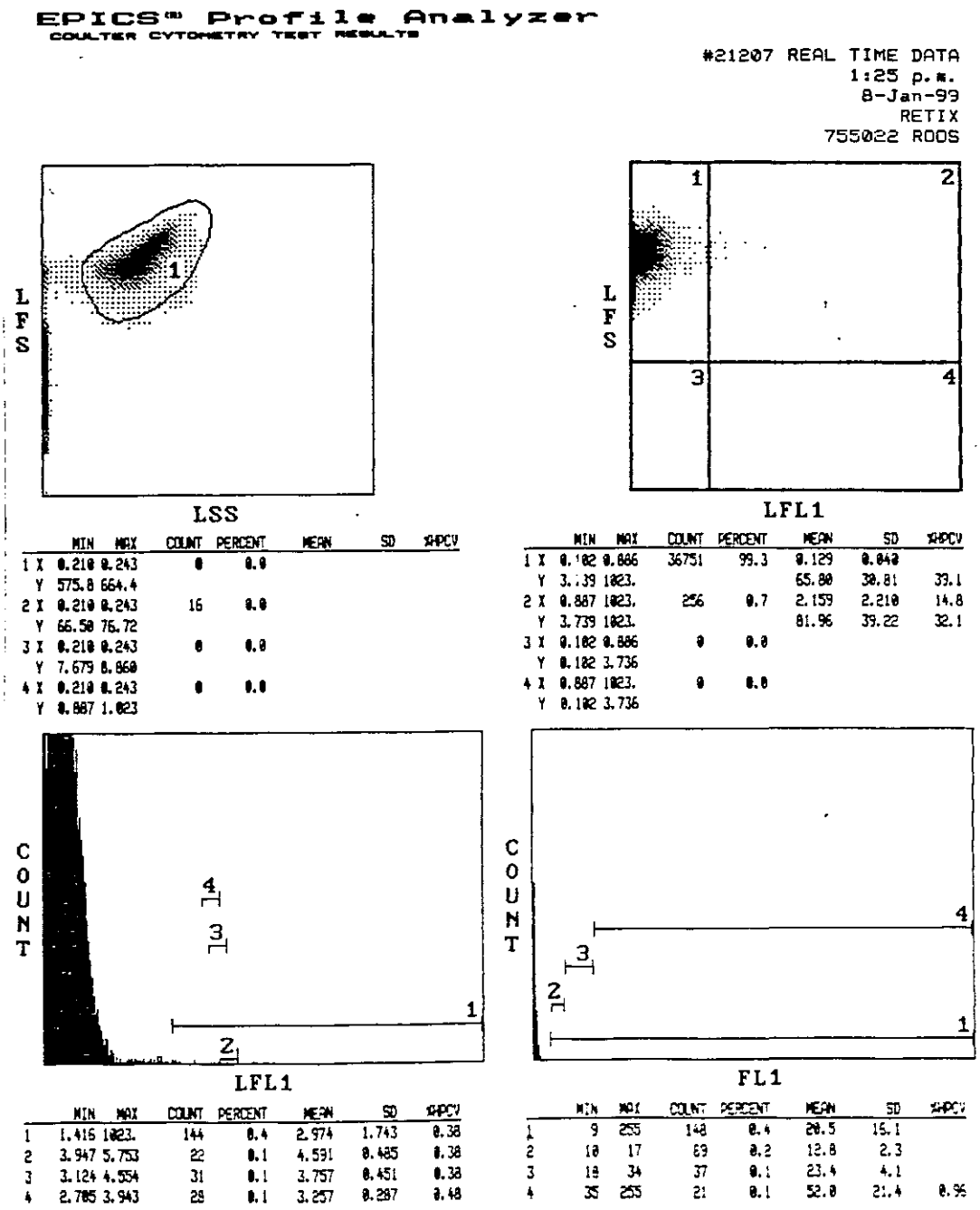
3.2.1. Instrument Protocol

The instrument was set to read 50 000 events. The red cell population was gated out, using log forward scatter and log side scatter. This was done because log parameters create a far tighter population group.

The gated information was then gated a second time on a log forward scatter and log fluorescence one bitmap. The use of a second gate allows for the removal of additional unwanted information, such as **DNA** stained granulocytes, lymphocytes and monocytes that could interfere with the red cell population resulting in false increased counts.

This gated information was finally plotted on a distribution histogram of two hundred and fifty five channels. The distribution histogram allowed for final elimination of mature erythrocytes from the reticulocyte population. This information was established on 100 male and female adults with normal full blood counts.

The reticulocyte population was divided into Low Fluorescing Reticulocytes (**LFR**), Medium Fluorescing Reticulocytes (**MFR**) and High Fluorescing Reticulocytes (**HFR**) within the normal distribution curve. Finally the Reticulocyte Maturation Index (**RMI**) was calculated from the raw counts obtained from the curve (Figure 2 Page 32).



		MIN	MAX	COUNT	PERCENT	MEAN	SD	%PCV
1	X	0.210	0.243	0	0.0			
	Y	575.8	664.4					
2	X	0.210	0.243	16	0.0			
	Y	66.58	76.72					
3	X	0.210	0.243	0	0.0			
	Y	7.679	8.860					
4	X	0.210	0.243	0	0.0			
	Y	0.887	1.023					

		MIN	MAX	COUNT	PERCENT	MEAN	SD	%PCV
1	X	0.102	0.886	36751	99.3	0.129	0.048	
	Y	3.739	1023.			65.80	30.81	33.1
2	X	0.887	1023.	256	0.7	2.159	2.210	14.8
	Y	3.739	1023.			81.96	39.22	32.1
3	X	0.102	0.886	0	0.0			
	Y	0.102	3.736					
4	X	0.887	1023.	0	0.0			
	Y	0.102	3.736					

	MIN	MAX	COUNT	PERCENT	MEAN	SD	%PCV
1	1.416	1023.	144	0.4	2.974	1.743	0.38
2	3.947	5.753	22	0.1	4.591	0.485	0.38
3	3.124	4.554	31	0.1	3.757	0.451	0.38
4	2.785	3.943	28	0.1	3.257	0.287	0.48

	MIN	MAX	COUNT	PERCENT	MEAN	SD	%PCV
1	9	255	148	0.4	20.5	15.1	
2	10	17	69	0.2	12.8	2.3	
3	18	34	37	0.1	23.4	4.1	
4	35	255	21	0.1	52.0	21.4	0.9%

Figure 2: A print out of reticulocyte analysis on an **EPICS Profile II** flow cytometer.
Region 1: Using log forward scatter and log side scatter, a dot plot of an erythrocyte population tight enough to bitmap was created.
Region 2: The information from region 1 is viewed against log fluorescence one, 99.9% autoflourescence was gated in quadrant one, the fluorescing reticulocytes were gated in quadrant 2.
Region 3: The information from region 2 is transferred onto a log histogram, because the channels in a log histogram are too difficult to place, these counts were not used, it was only placed as a clearer view the reticulocyte population.
Region 3: The information from Region 2 was transferred onto a histogram with 255 channels. Count 1 represents the total reticulocyte count and percentage, count 2 represents the **LFR** count and percentage, count 3 represents the **MFR** count and percentage, count 4 represents the **HFR** count and percentage.

3.2.2. Establishment of the Reticulocyte Maturation Index (RMI)

In 1993 Davis *et al* established the fluorescent intensity of reticulocytes into low **(LFR)**, medium **(MFR)** and high **(HFR)** intensity regions. On the Coulter **EPICS Profile II** normal samples fell between channel 1.30 and 2.00, the **LFR** events fell below 1.30 and the **HFR** above 2.00. When calculating the **RMI** a method using the number of reticulocytes in the **HFR** divided by the total number of reticulocytes yielded a reference range of 0.2 to 0.5 units.

A hundred patients with normal full blood counts were used to establish a reference range for Total Reticulocyte Percentage (>channel 10 < channel 255). A normal distribution was established and a Mean Channel Rate (**MCR**) was calculated. A two Standard Deviation (**2SD**) was then calculated from the information. Channels for Low Fluorescent Rate **LFR** (> channel 10 and < channel 18), Medium fluorescent Rate **MFR** (> channel 18 and > channel 35) and High Fluorescent Rate **HFR** (> channel 35 and < channel 255) were set on the instrument.

A further one hundred adults, with normal full blood counts, were then used to establish a normal range for the **LFR, MFR** and **HFR.** The Reticulocyte Maturation Index (**RMI**) could then be calculated (Figure 3 Page 33).

The **RMI** is the total number of cells counted in the **HFR** region divided by the number of cells counted in the Total Reticulocyte Percentage region, the results are represented as an International Unit (IU).

Reference Range

	Channels	Range
Total Reticulocyte %	>10 - <255	1.0 – 3.8 %
LFR	>10 - <18	0.2 – 0.8 %
MFR	>18 - <35	0.3 – 1.5 %
HFR	>35 - <255	0.1 – 1.5 %
RMI		0.18 – 0.50 IU

Figure 3: – Channels and established normal range for the Coulter **EPICS Profile II** flow cytometer at Groote Schuur Hospital.

3.2.3. The Control Group

The control number for the group was two hundred adults, both male and female with normal full blood counts.

3.3. Methodology

3.3.1. Reagent and Controls

- The reagent used for the study was Beckton Dickinson Retic-**COUNT**, commercially obtained Thiozole Orange (**TO**) fluorescent dye used to stain **RNA** and **DNA.**
- The controls were the Low, Normal and High reticulocyte count controls Retic-**CHEX,** commercially obtained from Streck Laboratories.

3.3.2. The Method

Five microlitres of test (**EDTA** blood) or control (Streck Retic-**CHECK**) were added to 500 microlitres of **TO** dye, incubated for one hour at room temperature and then analysed on the Coulter **EPICS Profile II** flow cytometer, the **RMI** was then calculated from the raw data count (Figure 2 Page 32).

3.4. The Data Collection

3.4.1. The Primary Data Collection

The primary data consisted of the calculated Reticulocyte Maturation Index (**RMI**). Each time a full blood count was taken on the post transplant patient, a reticulocyte analysis was done on the Coulter **EPICS Profile II** flow cytometer and recorded.

3.4.2. The secondary Data Collection

The secondary data consisted of the patients own or donor Mononuclear Count (**MNC**), Colony Forming Unit Granulocytic and Monocytic (**CFU-GM**) results, the Cluster Designation (**CD34+**) counts and the preconditioning protocol prior to transplant. The Absolute Neutrophil Count (**ANC**) and Platelet Count (**PLT**) were recorded each time a full blood count and differential count were requested. Finally, the bone marrow investigation results post transplant for "day 14", "day 28", three months, four months, five months, six months and one year post transplant were analysed.

3.4.3. The Population Sample

The population monitored were all Bone Marrow Transplant (**BMT**) and Peripheral Blood Stem Cell Transplant (**PBSCT**) patients from the Department of Haematology, Groote Schuur Hospital. Each patient in the study was monitored for six months post transplant; the one year bone marrow investigation was included in the analysis to establish the achievement of a "12 month post transplant trilineage response".

3.4.4. Statistical Analysis

All data was stored on Microsoft Excel spreadsheets. Calculations for mean, median and Standard Deviation (**SD**) were done using the package. All graphs were created using the information gathered from the spreadsheet. All the figures were done with Microsoft Word.

CHAPTER 4

Results

4.1. Introduction

Fifty one Bone Marrow Transplant (**BMT**) and Peripheral Blood Stem Cell Transplant (**PBSCT**) patients were examined. Each patient was monitored for six months post transplant. Although all patients were studied regardless of disease, two of the patients were removed from the study as they died prior to "day 28" post transplant. Ninety two percent of the patients engrafted within forty days, the remaining eight percent failed to engraft. The "graft failures" consisted of two Severe Aplastic Anaemia allogenic Bone Marrow Transplant (**SAA** allo**BMT**) patients, one Acute Myelocytic Leukaemia autologous Peripheral Blood Stem Cell Transplant (**AML** auto**PBSCT**) and a Severe Aplastic Anaemia allogenic Peripheral Blood Stem Cell Transplant (**SAA** allo**PBSCT**). These four patients were not used in the calculations. They were analysed separately.

Patients were divided into **BMT** and **PBSCT**, then further subdivided into allogenic and autologous transplants, and finally into disease types, these numbers grew considerably small (Figure 4 Page 38). Due to the fact that there was a substantial variety of disease types transplanted as well as three forms of transplants used, it was necessary to divide the patients into various groups to analyse outcomes individually as well as collectively. Eleven practical groups were established. Firstly analysis was done on "all transplants", with a total of forty five patients, and then division of the patients into thirty two "allogenic transplants', thirteen "autologous transplants", ten "**BMT**" and thirty five "**PBSCT**" was evaluated. Because the **PBSCT** transplants contained thirty five patients they could be analysed as twenty two "allo**PBSCT**" and thirteen "auto**PBSCT**". The allo**PBSCT** possessed sufficient numbers to subdivide further; eight "**AML** allo**PBSCT**" and seven "**CML** allo**PBSCT**" were evaluated. The nine "**AML** auto**PBSCT**" also made

up sufficient numbers for a separate analysis. A further group of twenty two patients that achieved a "12 month post transplant trilineage response" was analysed.

Each patient's Reticulocyte Maturation Index (**RMI**), Absolute Neutrophil Count (**ANC**) and Platelet Count (**PLT**) from day one until day forty, three months, four months, five months and six months were recorded on a Microsoft Excel spreadsheet. A mean Response Time, with a first to last day of response in brackets (Figure 5 Page 39) and median Response Time, with first to last day of response in brackets (Figure 6 Page 40) was calculated for each of the eleven groups.

A Point of Response was calculated in each peripheral blood parameter of the eleven groups by using Two Standard Deviation (**2SD**) statistical calculations, to establish a confidence limit. A 50%, 75% and 95% patient response within days of transplant were recorded (Figure 7 Page 41).

A percentage of patients who generated an **RMI**, **ANC** and **PLT** response prior to the mean were used to establish the effect of preconditioning on the bone marrow stroma. No statistical analysis was applied in an estimation of damage; it was only assumed that if more than fifty percent of the patients generated an early response, stromal damage was minimal.

The mean Mononuclear Count (**MNC**), Colony Forming Unit Granulocytic and Monocytic (**CFU-GM**), and Cluster Designation (**CD34+**) counts, pre transplant were determined on a Microsoft Excel spreadsheet for all eleven transplant groups (Figure 8 Page 44). The percentage of patients who generated **RMI**, **ANC** and **PLT** responses prior to the mean were compared to the **MNC**, **CFU-GM** counts greater than the mean. This comparison was used to establish the relationship between early parameter response and raised **MNC** and **CFU-GM** counts.

The mean **RMI**, **ANC** and **PLT** were used to calculate a **2SD** for the first forty days. These results were used to establish an expected Response Range (Figure 9 Page 45).

The final aspect of the analysis was to determine the mean and median **RMI**, **ANC** and **PLT** count at forty days, three months, four months, five months and six months post transplant. The mean (Figure 10 Page 46) and median **RMI** (Figure 11 Page 46) for six months was established and recorded. The mean (Figure 12 Page 47) and median **ANC** (Figure 13 Page 47) was established and recorded for six months. The mean (Figure 14 Page 48) and median **PLT** (Figure 15 Page 48) was established and recorded for six months. These counts for all three blood parameters throughout the first six months allowed us to establish a pattern of response for each group analysed.

4.1.1. Sample Population

A **BMT** or **PBSCT** was performed on each of the forty nine patients, and each transplant was either autologous or allogenic. The disease prior to transplant and how many patients in each group were recorded (Figure 4 Page 38):

Transplant	Type	Disease	n =
BMT	**Allo**	**ALL**	3
		CML	4
		AML	1
		NHL	1
		Myeloma	1
		SAA	2
	Auto		0
PBSCT	**Allo**	**AML**	8
		CML	7
		ALL	3
		LL	1
		Myeloma	1
		SAA	3
	Auto	**NHL**	2
		Stem	1
		AML	10
		BiPh	1
	Total		**49**

Figure 4 - Transplant Patients

4.1.2. Mean and Median Response Time for Reticulocyte Maturation Index (RMI), Absolute Neutrophil Count (ANC) and Platelet Count (PLT)

The data was analysed statistically using Microsoft Excel. The international criteria for **ANC** engraftment is 0.50 x10^9/l, 50x10^9/l for **PLT** engraftment and our own indication of 0.20 IU for **RMI** engraftment was used to establish a mean and the median response for all three parameters within the eleven groups. This was termed the "Response Time", which is used throughout the dissertation. If a patient was still alive after a year we termed it a "12 month post transplant trilineage response". This term is also used throughout the entire dissertation.

Figure 5 contains the results of the mean Response Time for the three peripheral blood parameters analysed, the number of patients in each group and the mean Response Time. The range of days from which a response was seen in each patient is in brackets.

Group	n =	Mean RMI	Mean ANC	Mean PLT
All TXT	45	**9** (1-17)	**16** (6-32)	**16** (1-36)
Allo TXT	32	**9** (1-17)	**16** (6-32)	**16** (1-36)
Auto TXT	13	**9** (1-17)	**15** (9-22)	**17** (10-28)
BMT	10	**8** (1-15)	**17** (6-32)	**17** (1-33)
PBSCT	35	**9** (1-17)	**15** (8-30)	**16** (7-36)
AlloPBSCT	22	**8** (1-17)	**13** (8-31)	**12** (7-36)
AutoPBSCT	13	**9** (1-17)	**15** (9-21)	**17** (10-28)
AML alloPBSCT	8	**7** (1-17)	**14** (8-30)	**14** (8-33)
CML alloPBSCT	7	**11** (5-16)	**20** (14-29)	**21** (7-36)
AML autoPBSCT	9	**10** (7-14)	**15** (9-21)	**18** (11-28)
12 month response	22	**8** (1-17)	**16** (6-32)	**15** (1-36)

Figure 5 - Mean Response Time and range for Reticulocyte Maturation Index (**RMI**), Absolute Neutrophil Count (**ANC**) and Platelet Count (**PLT**).

Figure 6 contains the results of the median Response Time for the three peripheral blood parameters analysed, the number of patients in each group and their median Response Time. The range of responses for each patient is seen in brackets.

Group	n =	Median RMI	Median ANC	Median PLT
All TXT	45	**9** (1-17)	**15** (6-32)	**14** (1-36)
Allo TXT	32	**9** (1-17)	**13** (6-32)	**12** (1-36)
Auto TXT	13	**10** (1-17)	**15** (9-22)	**15** (10-28)
BMT	10	**9** (1-15)	**15** (6-32)	**15** (1-33)
PBSCT	35	**9** (1-17)	**15** (8-30)	**14** (7-36)
AlloPBSCT	22	**8** (1-17)	**13** (8-31)	**12** (7-33)
AutoPBSCT	13	**10** (1-17)	**15** (9-21)	**15** (10-28)
AML alloPBSCT	8	**7** (1-17)	**13** (8-30)	**11** (8-36)
CML alloPBSCT	7	**12** (5-16)	**21** (14-29)	**21** (7-36)
AML autoPBSCT	9	**10** (7-14)	**15** (9-21)	**19** (11-28)
12 month response	22	**8** (1-17)	**14** (6-32)	**12** (1-36)

Figure 6 - Median Response Time and range for Reticulocyte Maturation Index (**RMI**), Absolute Neutrophil Count (**ANC**) and Platelet Count (**PLT**).

4.1.3. Point of Response for Reticulocyte Maturation Index (RMI), Absolute Neutrophil Count (ANC) and Platelet Count (PLT)

An **ANC** engraftment criteria of 0.50 $x10^9/l$ was used (Chin-yee *et al,* 1991;Davis *et al* 1992 + 1998;Greinix *et al* 1994;D'Onofrio *et al,* 1996). The **PLT** engraftment criteria was 50 $x10^9/l$ (Chin-yee *et al,* 1991;Davis *et al,* 1992). The **RMI** engraftment criteria was set at 0.20 IU, the bottom of the normal range. Blood was not collected every day for each patient in the study period. It was necessary to determine the response for each patient by using the above criteria. The **2SD** established which patients, fell within 95% of the time creating a confidence limit. This limit for engraftment was termed "Point of Response" and is used throughout the dissertation.

Figure 7 contains the mean Point of Response at 50%, 75% and 95% in all three parameters, **RMI**, **ANC** and the **PLT**, in all eleven groups. Some patients responded outside the 95% limit and later than forty days. These patients were not used in the calculations, but were noted and discussed within each group analysis.

		n =	50%	75%	95%
All TXT	**RMI**	45	9	12	16
	ANC	44	14	18	29
	PLT	43	14	20	33
Allo TXT	**RMI**	32	8	10	15
	ANC	31	14	20	30
	PLT	30	12	20	33
Auto TXT	**RMI**	13	10	13	16
	ANC	13	14	16	21
	PLT	13	15	21	24
BMT	**RMI**	10	9	10	15
	ANC	10	13	25	32
	PLT	8	13	29	33
PBSCT	**RMI**	35	9	12	16
	ANC	34	13	18	21
	PLT	35	14	21	33
AlloPBSCT	**RMI**	22	8	10	16
	ANC	21	14	18	29
	PLT	22	11	18	33
AutoPBSCT	**RMI**	13	10	13	16
	ANC	13	14	16	21
	PLT	13	15	21	24
AML alloPBSCT	**RMI**	8	6	9	17
	ANC	8	12	13	30
	PLT	8	10	14	32
CML alloPBSCT	**RMI**	7	12	14	16
	ANC	6	19	20	28
	PLT	7	21	28	36
AML autoPBSCT	**RMI**	9	11	13	14
	ANC	9	15	16	21
	PLT	9	19	21	23
12 month response	**RMI**	22	8	12	14
	ANC	22	14	18	26
	PLT	22	14	24	33

Figure 7 – Indication of days at which 50%, 75% and 95% of the patients reached their Point of Response.

4.1.4. Preconditioning

Different combinations of preconditioning were used depending on patient disease. Applying the mean Response Time, a percentage of patients who responded prior to the mean in the **RMI**, **ANC** and **PLT** parameters were calculated. Patients that responded prior to the mean were considered an "early response"; this term is used throughout the entire dissertation. In preconditioning where more than fifty percent of the patients generated early responses, it was assumed that they sustained very little stromal damage. Of those patients, if more than fifty percent of them achieved a "12 month post transplant trilineage response"; the assumption was that medium term recovery was positive, resulting in a confident projection of results.

Listed below is the preconditioning that was used on the patients within the study:

1. Bu/Cy (busulphan/Cyclophosphamide)
Busulphan = 4mg/kg or only x 4 days.

Cyclophosphamide = 60mg/kg ivi x 2days.

2. Cyclo/TBI/TNI (Cyclophosphamide + Total Body Irradiation and Total Nodal Irradiation).
Cyclophosphamide = 60mg/kg x 2 days.

The source of irradiation was a Cobalt 60 Instrument, which emitted gamma rays.

Total Body Irradiation = 2 Gy twice daily for 3 days.

Total Nodal Irradiation = 1.5 Gy twice daily for 2 days.

3. Cyclo/Melphalan/TBI/TNI (For Myeloma patients)
Cyclophosphamide = 400mg/m^2 ivi x 1 day.

Melphalan = 140mg/m^2 ivi x 1 day.

Total Body Irradiation = 2 Gy twice a day for 3 days.

Total Nodal Irradiation = 1.5 Gy twice a day for 2 days.

4. Cyclo/TNI (SAA, Standard adult protocol for T-cell depleted allograft)
Cyclophosphamide = 50mg/kg ivi x4 days.

Total Nodal Irradiation = 1.5 Gy twice a days for 6 days.

5. Cyclo/Alg (SAA, for non T-cell depleted transplants)
Cyclophosphamide = 50mg/kg ivi x 4days.

Anti lymphocyte globulin = 30mg/kg daily ivi for the first 3 days of Cyto.

6. Cyclo/TNI/Campath (SAA, old aplastic protocol using in vivo campath)
Cyclophosphamide = 50mg/kg 1 dose x 4 days.

Total Nodal Irradiation = 1.5 Gy twice a day for 6 days.

Campath IG = 10mg ivi daily for the last 4 days of **TNI**.

7. Bu/Mel/Thiothepa
Busulphan = 4mg/kg daily x 3days.

Melphalan = 50mg/m^2 daily x 2 days.

Thiothepa = 150mg/m^2 daily x 2 days

4.1.5. Mononuclear Count (MNC), Colony Forming Unit-Granulocytic and Monocytic Count (CFU-GM) and Cluster Designation 34+ Counts (CD34+)

The **MNC** and **CFU-GM** were determined prior to transplant on all the patients. A mean (Figure 8 Page 44) was established on a Microsoft Excel spreadsheet for both **MNC** and the **CFU-GM**. **CD34+** counts were only accurately performed on the last seven patients in the study. Although they were reported, their findings are inconclusive, further studies would be required for an accurate analysis.

If more than fifty percent of the patients obtained a **MNC** or **CFU-GM** count greater than the mean and an "early response" in any of the three peripheral blood parameters (**RMI**, **ANC** or **PLT**), it was assumed that higher counts recognised a direct impact on early response. If more than fifty percent of them achieved a "12 month post transplant trilineage response"; they were considered to have experienced a positive medium term outcome, as a result of the higher counts.

Figure 8 contains the mean **MNC, CFU-GM** and **CD34+** counts prior to transplant for the eleven groups analysed.

Transplant	n =	MNC INF X 10^8/kg	No of Pt	CFU-GM X 10^4/kg	No of Pt	CD34+
All TXT	**47**	6.12	**36**	26.10	**7**	83.83
AlloTXT	**34**	5.83	**28**	26.95	**5**	62.35
Auto TXT	**14**	6.53	**8**	22.15	**2**	137.55
BMT	**10**	0.97	**8**	16.75	**0**	
PBSCT	**37**	7.51	**28**	28.77	**7**	83.83
AlloPBSCT	**24**	7.93	**20**	31.25	**5**	62.35
AutoPBSCT	**13**	6.53	**8**	22.15	**2**	137.55
AML alloPBSCT	**8**	7.96	**7**	18.15	**1**	34.22
CML alloPBSCT	**7**	6.69	**4**	22.21	**2**	53.78
AML autoPBSCT	**9**	5.97	**6**	27.84	**2**	137.55
12 month response	**22**	5.83	**16**	19.84	**3**	113.68

Figure 8 – All the **MNC INF, CFU-GM** and **CD34+** means for the transplant patients.

4.1.6. Expected Response Range

Once the mean Response Time had been calculated, a **2SD** was established to create an expected Response Range. The Response Range was calculated from day one to day forty, three months, four months, five and six months. This enabled a guideline for daily response post transplant to be established. A percentage of patients who exceeded or fell short of **2SD** in all three blood parameters was determined, as well as what percentage of them achieved a "12 month post transplant trilineage response". In turn this allowed for evaluation of "graft failures" in relation to **2SD** during the first forty days.

Figure 9 contains the mean **RMI, ANC** and **PLT** counts (highlighted) and the **2SD** range (in brackets) for the fist forty days, three months, four months, five months and six months.

Expected Response Range

Days	RMI Mean/range	ANC Mean/range	PLT Mean/range
1	**0.17** (0.02-0.32)	**0.43** (0.00-1.82)	**55** (0-102)
2	**0.19** (0.04-0.35)	**0.01** (0.00-0.02)	**52** (0-170)
3	**0.15** (0.00-0.33)	**0.40** (0.00-2.47)	**44** (0-133)
4	**0.12** (0.05-0.19)	**0.00** (0.00-0.00)	**30** (0-100)
5	**0.12** (0.00-0.24)	**0.03** (0.00-0.11)	**38** (0-95)
6	**0.18** (0.04-0.33)	**0.27** (0.00-1.94)	**26** (0-61)
7	**0.17** (0.02-0.31)	**0.06** (0.00-0.33)	**31** (0-70)
8	**0.25** (0.00-0.55)	**0.18** (0.00-1.23)	**33** (0-72)
9	**0.23** (0.09-0.36)	**0.09** (0.00-0.39)	**39** (0-92)
10	**0.25** (0.02-0.49)	**0.44**(0.00-1.86)	**35** (0-79)
11	**0.30** (0.07-0.53)	**0.26** (0.00-0.98)	**35** (0-67)
12	**0.27** (0.05-0.48)	**0.51** (0.00-1.85)	**39** (0-87)
13	**0.35** (0.02-0.67)	**0.91** (0.00-2.87)	**40** (0-103)
14	**0.28** (0.07-0.48)	**0.67** (0.00-2.55)	**47** (0-103)
15	**0.32** (0.04-0.61)	**1.84** (0.00-8.25)	**49** (0-91)
16	**0.34** (0.00-0.67)	**0.52** (0.00-1.63)	**29** (0-66)
17	**0.32** (0.10-0.55)	**1.36** (0.00-5.02)	**46** (0-98)
18	**0.33** (0.09-0.57)	**0.47** (0.00-1.23)	**34** (0-69)
19	**0.34** (0.16-0.52)	**0.88** (0.00-2.28)	**33** (0-62)
20	**0.32** (0.08-0.56)	**1.45** (0.00-3.48)	**39** (0-125)
21	**0.31** (0.08-0.54)	**1.61** (0.00-4.87)	**42** (0-110)
22	**0.34** (0.10-0.59)	**1.60** (0.00-4.30)	**41** (0-99)
23	**0.30** (0.00-0.65)	**1.81** (0.00-1.81)	**28** (0-41)
24	**0.29** (0.03-0.50)	**1.72** (0.00-4.92)	**53** (0-90)
25	**0.24** (0.08-0.40)	**1.04** (0.00-4.23)	**38** (0-90)
26	**0.27** (0.02-0.52)	**0.96** (0.00-2.94)	**47** (0-124)
27	**0.37** (0.17-0.57)	**1.01** (0.00-2.28)	**29** (0-57)
28	**0.32** (0.09-0.56)	**0.84** (0.00-2.26)	**91** (0-204)
29	**0.34** (0.13-0.54)	**0.98** (0.00-2.34)	**69** (0-152)
30	**0.28** (0.07-0.50)	**0.10** (0.00-0.10)	**52** (0-52)
31	**0.31** (0.04-0.56)	**3.12** (0.00-11.16)	**34** (0-74)
32	**0.37** (0.08-0.62)	**0.75** (0.00-2.02)	**43** (0-94)
33	**0.35** (0.28-0.44)	**1.44** (0.00-3.38)	**64** (0-148)
34	**0.37** (0.03-0.72)	**2.61** (0.00-8.10)	**84** (0-197)
35	**0.40** (0.04-0.76)	**1.63** (0.00-4.37)	**54** (0-128)
36	**0.48** (0.36-0.60)	**2.88** (0.00-9.35)	**72** (0-112)
37	**0.44** (0.19-0.69)	**0.87** (0.00-0.87)	**42** (7-87)
38	**0.43** (0.24-0.61)	**2.99** (0.00-7.21)	**36** (0-99)
39	**0.37** (0.14-0.60)	**1.45** (0.00-2.86)	**60** (0-115)
40	**0.41** (0.26-0.50)	**1.94** (0.00-3.95)	**76** (0-100)
3 Months	0.29 (0.08-0.49)	**2.25** (0.00-6.59)	**91** (0-173)
4 Months	0.25 (0.08-0.43)	**2.27** (0.00-6.17)	**120** (1-240)
5 Months	0.20 (0.04-0.36)	**2.18** (0.00-4.56)	**138** (0-282)
6 Months	0.17 (0.00-0.38)	**2.92** (0.00-6.82)	**164** (0-308)

Figure 9 - Mean of all forty five patients with a Two Standard Deviation **(2SD).**

4.1.7. Medium Term Mean and Median Counts

Mean and median counts for each parameter was calculated, at forty days, three months, four months, five months and six months, establishing a percentage increase or decrease for each group from forty days to six months. This allowed for estimated response and a recovery over a longer period of time.

Figure 10 contains the mean **RMI** value for the eleven groups from forty days to six months, and the percentage decrease over that period.

Mean RMI	40 days	3 months	4 months	5 months	6 months	Decrease
All TXT	0.39	0.26	0.26	0.21	0.22	77%
Allo TXT	0.37	0.24	0.23	0.21	0.26	42%
Auto TXT	0.41	0.30	0.31	0.21	0.16	156%
BMT	0.45	0.25	0.27	0.23	0.33	36%
PBSCT	0.38	0.27	0.26	0.20	0.19	100%
AlloPBSCT	0.33	0.23	0.22	0.19	0.22	50%
AutoPBSCT	0.41	0.30	0.31	0.21	0.16	156%
AML alloPBSCT	0.33	0.25	0.19	0.18	0.17	94%
CML alloPBSCT	0.45	0.22	0.26	0.21	0.28	60%
AML autoPBSCT	0.40	0.29	0.29	0.21	0.18	122%
12 month response	0.41	0.29	0.25	0.20	0.17	141%

Figure 10 – Mean Reticulocyte Maturation Index (**RMI**) results for transplants at forty days, three months, four months, five months, six months and percentage decrease.

Figure 11 contains the median **RMI** values for the eleven groups from forty days to six months including the percentage decrease for that period of time.

Median RMI	40 days	3 months	4 months	5 months	6 months	Decrease
All TXT	0.40	0.26	0.27	0.21	0.22	82%
Allo TXT	0.33	0.21	0.26	0.20	0.26	27%
Auto TXT	0.40	0.33	0.32	0.21	0.16	150%
BMT	0.45	0.25	0.31	0.23	0.36	25%
PBSCT	0.39	0.26	0.26	0.20	0.18	117%
AlloPBSCT	0.33	0.21	0.23	0.19	0.22	50%
AutoPBSCT	0.40	0.33	0.32	0.21	0.16	150%
AML alloPBSCT	0.33	0.22	0.21	0.19	0.17	94%
CML alloPBSCT	0.45	0.20	0.26	0.20	0.33	36%
AML autoPBSCT	0.40	0.33	0.32	0.21	0.16	150%
12 month response	0.45	0.30	0.23	0.19	0.16	181%

Figure 11 – Median Reticulocyte Maturation Index (**RMI**) results for transplants at forty days, three months, four months, five months, six months and percentage decrease.

The mean ANC for each of the eleven groups at forty days, three months, four months, five months and six months was calculated

Figure 12 contains the mean ANC values for the eleven groups from forty days to six months, including the percentage increase for that time period.

Mean ANC	40 days	3 months	4 months	5 months	6 months	Increase
All TXT	1.63	2.51	2.44	2.09	2.77	70%
Allo TXT	1.63	2.30	2.05	2.18	2.64	62%
Auto TXT	1.12	2.89	3.20	1.90	3.03	171%
BMT	2.65	2.20	2.01	2.15	2.64	0%*
PBSCT	1.13	2.60	2.57	2.06	2.82	150%
AlloPBSCT	1.13	2.36	2.07	2.19	2.64	134%
AutoPBSCT	1.12	2.89	2.30	1.90	3.03	171%
AML alloPBSCT	1.13	3.10	2.41	1.62	2.78	146%
CML alloPBSCT	0.12	1.69	1.87	1.56	2.52	2000%
AML autoPBSCT	1.12	2.09	2.70	1.71	2.91	160%
12 month response	1.94	2.25	2.27	2.18	2.92	51%

Figure 12 – Mean Absolute Neutrophil Count (ANC) results for transplants at forty days, three months, four months, five months, six months and percentage increase. *Indicates a decrease in value from forty days to six months.

Figure 13 contains the median ANC values for the eleven groups from forty days to six months, including the percentage increase for the time period.

Median ANC	40 days	3 months	4 months	5 months	6 months	Increase
All TXT	1.23	2.10	1.94	1.78	2.43	98%
Allo TXT	1.23	2.44	1.89	1.78	2.28	85%
Auto TXT	1.30	1.54	2.24	1.78	2.88	122%
BMT	2.63	1.53	1.87	2.09	2.28	15%*
PBSCT	1.13	2.25	2.03	1.79	2.61	131%
AlloPBSCT	1.13	2.68	1.90	1.52	2.31	104%
AutoPBSCT	1.30	1.54	2.24	1.78	2.88	122%
AML alloPBSCT	1.13	2.87	1.67	1.45	2.05	81%
CML alloPBSCT	0.12	0.92	0.94	1.38	2.85	2275%
AML autoPBSCT	1.30	1.28	1.71	1.78	2.78	114%
12 month response	1.94	1.76	1.90	1.78	2.28	18%

Figure 13 – Median Absolute Neutrophil Count (ANC) results for transplants at forty days, three months, four months, five months, six months and percentage increase. * Indicates a decrease in value from forty days to six months.

The mean **PLT** for forty days, three months, four months, five months and six months was calculated.

Figure 14 contains the mean **PLT** for the period of forty days to six months, including the percentage increase for the time period.

Mean PLT	40 days	3 months	4 months	5 months	6 months	Increase
All TXT	60	99	120	127	141	135%
Allo TXT	58	116	137	140	150	159%
Auto TXT	40	64	77	92	115	188%
BMT	78	116	120	133	137	76%
PBSCT	44	91	115	120	137	211%
AlloPBSCT	48	115	147	145	157	227%
AutoPBSCT	40	64	77	92	115	188%
AML alloPBSCT	48	123	152	111	133	177%
CML alloPBSCT	64	87	128	140	141	120%
AML autoPBSCT	49	123	152	111	133	171%
12 month response	76	91	120	138	164	116%

Figure 14 – Mean Platelet Count (**PLT**) results for transplants at forty days, three months, four months, five months, six months and percentage increase.

Figure 15 contains the median **PLT** for the period of forty days to six months, including the percentage increase for the time period.

Median PLT	40 days	3 months	4 months	5 months	6 months	Increase
All TXT	71	95	110	120	132	86%
Allo TXT	78	109	141	126	161	106%
Auto TXT	50	70	72	73	107	114%
BMT	78	109	112	120	132	69%
PBSCT	40	78	96	119	127	218%
AlloPBSCT	48	107	160	126	165	244%
AutoPBSCT	40	70	72	73	107	168%
AML alloPBSCT	48	147	178	115	163	240%
CML alloPBSCT	64	93	141	126	161	152%
AML autoPBSCT	36	147	178	115	163	353%
12 month response	78	93	102	119	132	69%

Figure 15 – Median Platelet Count (**PLT**) results for transplants at forty days, three months, four months, five months, six months and percentage increase.

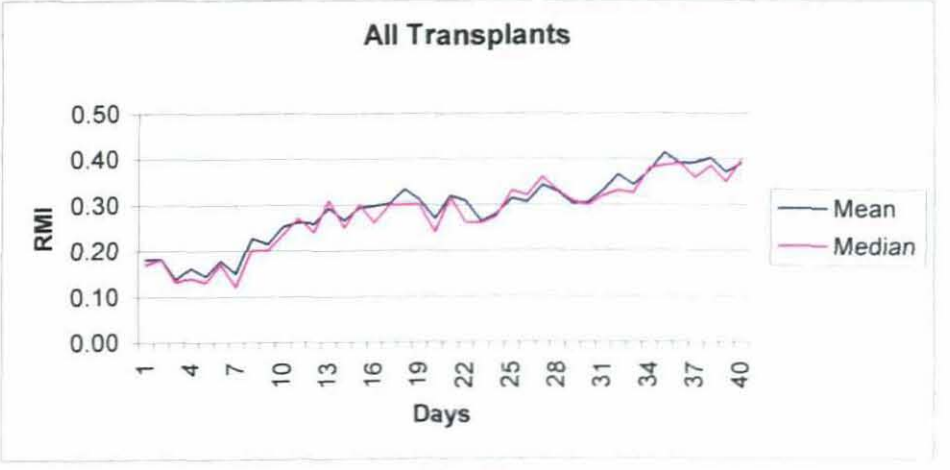
4.2. Comparison of Transplants

Forty five patients were analysed in this group. Of the original forty nine patients two **SAA** allo**BMT** were excluded, one **SAA** allo**PBSCT** who failed the first transplant but achieved a successful second and one auto**PBSCT AML** who failed the transplant.

4.2.1. The Reticulocyte Maturation Index (RMI) Response

4.2.1.1. The Mean and Median Response Time for the Reticulocyte Maturation Index (RMI) on Transplant Patients

Both the mean (Figure 5 Page 39) and the median (Figure 6 Page 40) Response Time was nine days, with a range of one to seventeen days. One hundred percent patient response was seen within seventeen days.



Graph 1: The mean and median **RMI** Response Time, in days, for forty five transplant patients.

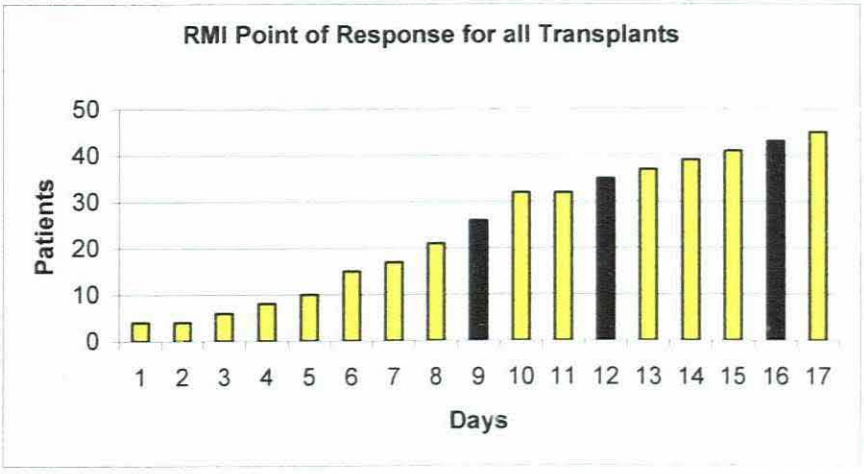
A mean **RMI** of 0.39 IU was seen within forty days (Graph 1 Page 49), decreasing to 0.26 IU within three months, 0.26 IU at four months, 0.21 IU at five months and 0.22 IU at six months, a seventy seven percent mean decrease (Figure 10 Page 46). Although the values remained within the normal range (0.20 - 0.50 IU) for six months post transplant, they constantly ran in the lower limits of normal.

A median **RMI** of 0.40 IU was seen within forty days (Graph 1 Page 49), 0.26 IU at three months, 0.27 IU at four months, 0.21 IU at five months and 0.22 IU at six months, an eighty two percent median decrease (Figure 11 Page 46).

The **RMI** was 0.39 IU at forty days, and 0.22 IU at six months (a seventy seven percent decrease). This was possibly due to an initial response to anaemia, with a stabilisation of erythroid production later.

4.2.1.2. The Point of Response for the Reticulocyte Maturation Index (RMI) on Transplant Patients

A 95% Point of Response was seen within sixteen days, 50% within nine days and 75% within twelve days (Graph 2 Page 50).



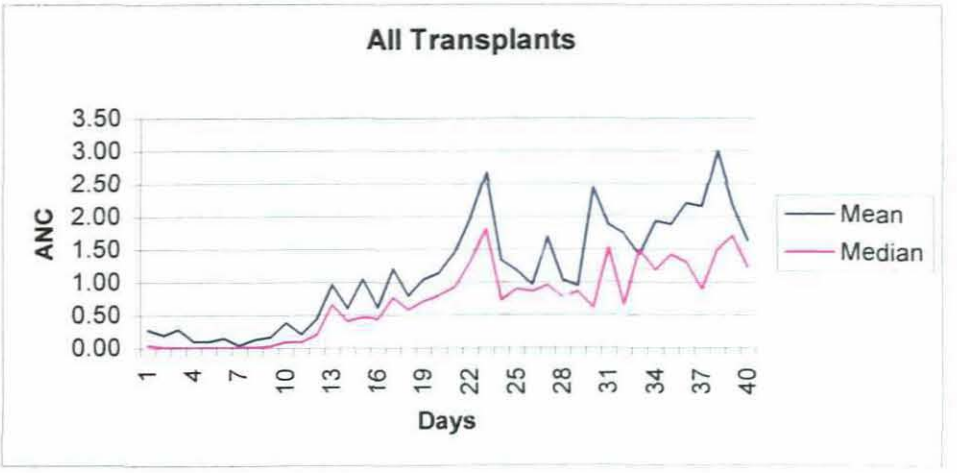
Graph 2: The **RMI** Point of Response, in days, for forty five patients, 50%, 75% and 95% are highlighted.

Ninety six percent of the patients fell within 95% Point of Response. Two patients, an **AML** allo**PBSCT** and a **CML** allo**PBSCT** responded on day seventeen, they were not considered "delayed responses".

4.2.2. The Absolute Neutrophil Count (ANC) Response

4.2.2.1. The Mean and Median Response Time for the Absolute Neutrophil Count (ANC) on Transplant Patients

The mean Response Time was sixteen days (Figure 5 Page 39) and the median Response Time was fifteen days (Figure 6 Page 40), with a range of six to thirty two days. One hundred percent patient response was seen within thirty two days.



Graph 3: The mean and median **ANC** Response Time, in days, for forty four transplant patients.

A mean **ANC** of 1.63 $x10^9$/l was seen within forty days (Graph 3 Page 51), 2.51 $x10^9$/l at three months, 2.44 $x10^9$/l at four months, 2.09 $x10^9$/l at five months and 2.77 $x10^9$/l at six months, a seventy percent mean increase (Figure 12 Page 47). The **ANC** increased over six months, where the **RMI** decreased. Although the **ANC** count was within the normal range (1.8 - 7.7 $x10^9$/l) for most of the time the results still remained within the lower limits of normal.

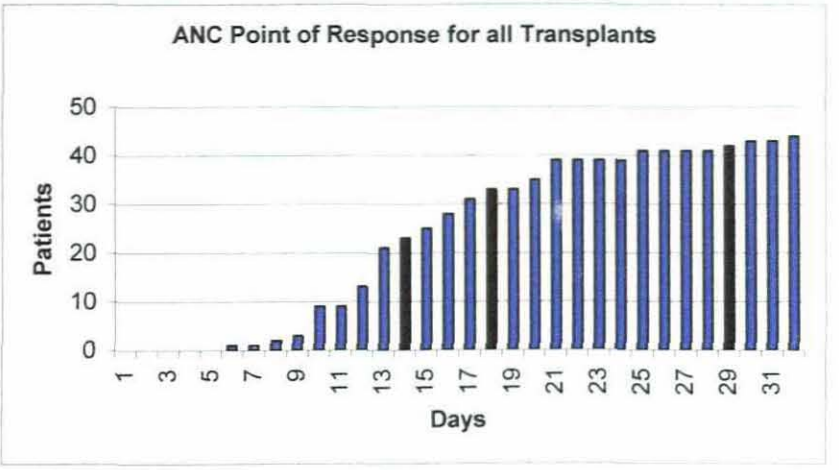
A median **ANC** of 1.23 $x10^9$/l was seen within forty days (Graph 3 Page 51), 2.10 $x10^9$/l at three months, 1.94 $x10^9$/l at four months, 1.78 $x10^9$/l at five months and 2.43 $x10^9$/l at six months, a ninety eight percent median increase (Figure 13 Page 47).

A mean **RMI** response was seven days earlier than the **ANC** response, indicating an earlier erythroid response.

The **ANC** count at forty days was 1.63 $x10^9$/l and 2.77 $x10^9$/l at six months. The **ANC** count had a seventy percent increase compared to the **RMI** which had a seventy percent decrease.

4.2.2.2. The Point of Response for the Absolute Neutrophil Count (ANC) on Transplant Patients

A 95% Point of Response was seen within twenty nine days, 50% within fourteen days and 75% within eighteen days (Graph 4 Page 52).



Graph 4: The **ANC** Point of Response, in days, for forty two patients, 50%, 75% and 95% limits are highlighted.

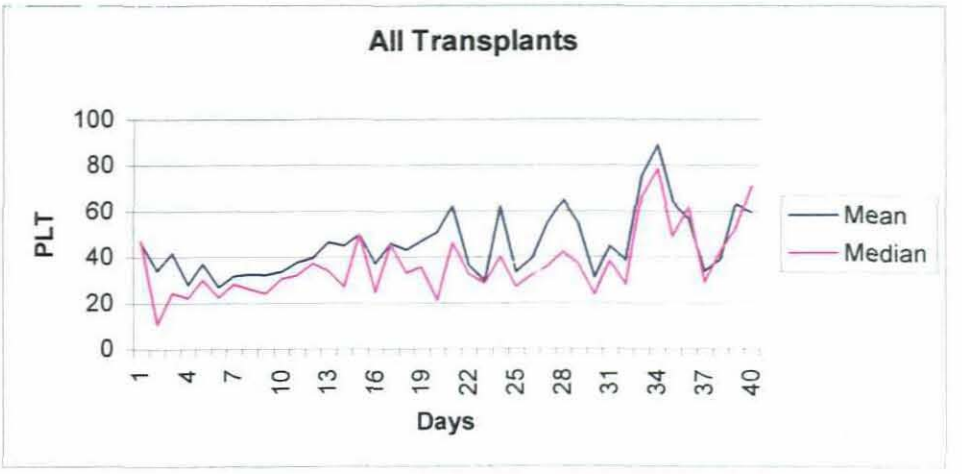
Ninety eight percent of the patients responded within 95% Point of Response. Two patients fell outside the limit, one an **AML** allo**PBSCT** who responded on day thirty and the other a **NHL** allo**BMT** who responded on day thirty two. Neither were considered a "delayed response", only the **NHL** allo**BMT** achieved a "12 month post transplant trilineage response". One patient was not used in the calculation, a **CML** allo**PBSCT** who only responded on day fifty seven. She was considered a "failed response", although a "12 month post transplant trilineage response" was achieved.

The **RMI** Point of Response was seen thirteen days prior to the **ANC** response, indicating an earlier erythroid response.

4.2.3. The Platelet Count (PLT) Response

4.2.3.1. *The Mean and Median Response Time for the Platelet Count (PLT) on Transplant Patients*

The mean Response Time was sixteen days (Figure 5 Page 39) and the median Response Time was fourteen days (Figure 6 Page 40), with a range of one to thirty six days. A ninety six percent patient response was seen within thirty six days.



Graph 5: The mean and median **PLT** Response Time, in days, for forty three transplant patients.

A mean **PLT** of 60 x10^9/l was seen within forty days (Graph 5 Page 53), 99 x10^9/l at three months, 120 x10^9/l at four months, 127 x10^9/l at five months and 141 x10^9/l at six months, a one hundred and thirty five percent mean increase (Figure 14 Page 48). The **PLT** like the **ANC** revealed a steady increase of counts over six months, although the values remained within the lower limits of normal (150 – 450 x10^9/l).

A median **PLT** of 71 x10^9/l was seen within forty days (Graph 5 Page 53), 95 x10^9/l at three months, 110 x10^9/l at four months, 120 x10^9/l at five months and 132 x10^9/l at six months, an eighty six percent median increase (Figure 15 Page 48).

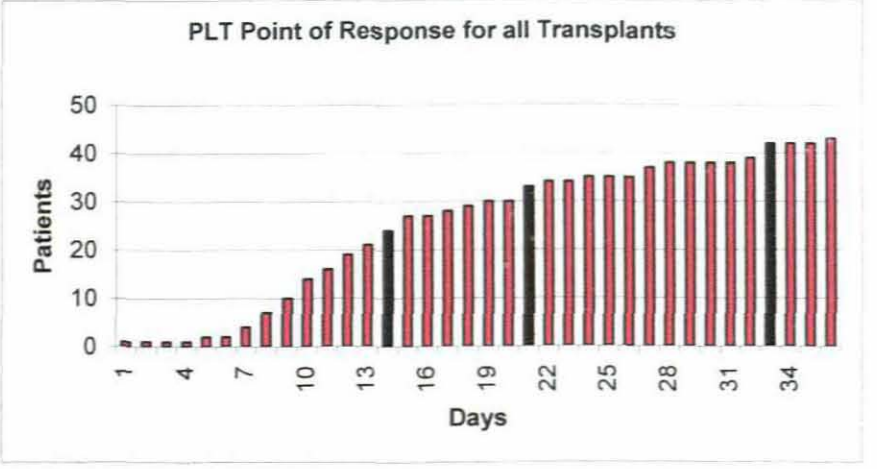
The mean **RMI** response was seven days prior to both the **ANC** and **PLT**, indicating an earlier erythroid response.

The **PLT** count at forty days was 60 $x10^9$/l and 141 $x10^9$l at six months (a one hundred and thirty five percent increase). The **PLT** had the best recovery rate of all three, possibly due to a faster return of megakaryocytic production.

Graphical representation of the three parameters indicated that the **RMI** mean and the median results were Gaussian (Graph 1 Page 49). The **ANC** (Graph 3 Page 51) and **PLT** (Graph 5 Page 53) were not Gaussian. Although once the **RMI** and the **ANC** reached their required Response Time, they climbed steadily to reach acceptable limits within the first forty days. The **RMI** did, however, show a far steadier increase and maintenance of numbers. The **ANC** revealed more fluctuations than the **RMI,** despite fluctuations they remained above the lower limits of response. The **PLT** fluctuations continued to be unstable, they rose and fell above and below the 50 $x10^9$/l mark possibly due to the frequency of platelet transfusions.

4.2.3.2. The Point of Response for the Platelet Count (PLT) on Transplant Patients

A 95% Point of Response was seen within thirty three days, 50% within fourteen days and 75% within twenty days (Graph 6 Page 54).



Graph 6: The **PLT** Point of Response for Transplants, in days, for forty two patients, 50%, 75% and 95% limits are highlighted.

Ninety three percent of the patients responded within 95% Point of Response. One patient a **CML** allo**PBSCT** had a "slightly delayed response" at thirty six days, she was the same patient with an **ANC** "delayed response". Two patients, both **CML** allo**BMT**

responded on day forty six and seventy three respectively, they were both considered a "delayed response". Both fell within the 95% limits for the **RMI** and **ANC,** both relapsed and died. The **PLT** response was the least stable of the three; it appeared that "slightly delayed" **PLT** responders did have a better chance of success than the "true delayed responses".

The **RMI** Point of Response was seen seventeen days prior to the **PLT** response and four days earlier than the **ANC** response. This indicated an earlier **RMI** response and reiterated the importance of the **RMI** response as an engraftment tool.

4.2.4. Preconditioning in Transplant Patients

Forty nine preconditioning regimes were used, the treatment was as follows:

Thirty one patients were preconditioned with **Cyclo/TBI/TNI**.

- Ten **BMT**.
- Seven **AML** allo**PBSCT**.
- Five **CML** allo**PBSCT**.
- Three **ALL** allo**PBSCT**.
- One lymphocytic lymphoma allo**PBSCT**.
- Four **AML** auto**PBSCT**.
- One biphenotypic auto**PBSCT**.

Nine patients were preconditioned with **Bu/Cy**.

- One **AML** allo**PBSCT**.
- Two **CML** allo**PBSCT**.
- One stem cell leukaemia auto**PBSCT**.
- Five **AML** auto**PBSCT**.

Four patients were preconditioned with **Bu/Mel/Thiothep**.

- One allo**PBSCT** myeloma.
- Two **NHL** auto**PBSCT** patients.

- One **AML** auto**PBSCT** patient.

Four patients were preconditioned with **Cyclo/TNI**.

- Both the **SAA** allo**BMT**.
- Two of the **SAA** allo**PBSCT**.

One patient was preconditioned with **Cyclo/Alg**.

- One of the **SAA** allo**PBSCT**.

Fifty nine percent of the patients were preconditioned with **Cyclo/TBI/TNI**, forty percent of them achieved a "12 month post transplant trilineage response". Seventy three percent of the patients treated with **Cyclo/TBI/TNI** produced an early **RMI** response; forty five percent of them achieved a "12 month post transplant trilineage response". Seventy percent of the patients produced an early **ANC** response; forty eight percent of them achieved a "12 month post transplant trilineage response". Sixty seven percent of the patients produced an early **PLT** response; forty percent of them achieved a "12 month post transplant trilineage response". With more than fifty percent of the patients generating early responses in all three parameters, this indicated stromal damage as a result of **Cyclo/TBI/TNI** was minimal. Because less than fifty percent of them achieved a "12 month post transplant trilineage response, the possibility of a projected medium term success was less positive.

Eighteen percent of the patients were preconditioned with **Bu/Cy**; fifty six percent of them achieved a "12 month post transplant trilineage response". Twenty two percent of the patients produced an early **RMI** response, one died. Forty four percent of the patients produced an early **ANC** response; fifty percent achieved a "12 month post transplant trilineage response". Forty four percent of the patients produced an early **PLT** response; seventy five percent achieved a"12 month post transplant trilineage response". Although the numbers were low, less than fifty percent of the patients generated early blood parameter response, the appearance of some stromal damage as a result of **Bu/Cy** was indicated. The projected medium term success was even less positive. The

megakaryocytic damage appeared to be lower than the erythroid or granulocytic damage, as more than fifty percent achieved a "12 month post transplant trilineage response".

Of the four patients treated with **Bu/Mel/Thiotep**, two died. Early responses were seen in one of the patients who achieved a "12 month post transplant trilineage response". These numbers were too low for accurate evaluation.

Two **SAA** allo**PBSCT** patients were treated with **Cyclo/TNI**, although they both achieved a "12month post transplant trilineage response", only one generated early responses in all three parameters, and the other only in the **PLT**. The two **SAA** allo**BMT** treated with **Cyclo/TNI** both died. One had early **RMI** and **PLT** responses, the other not. The **SAA** allo**PBSCT** treated with **Cyclo/Alg** showed no early response in any of the parameters, but achieved a "12 month post transplant trilineage response". The numbers again were too low for accurate evaluation.

4.2.5. Mononuclear Count (MNC), Colony Forming Unit - Granulocytic Monocytic (CFU-GM) and Cluster Designation 34+ Count (CD34+) in Transplant Patients

All the patients were evaluated for **MNC**, with a mean of 6.12 $x10^8$/kg (including the **BMT**). Seventy six percent of the patients were evaluated for **CFU-GM**, with a mean of 26.10 $x10^4$/kg, eight percent were contaminated with bacteria and sixteen percent had resulted in no growth. The **CD34+** mean was 83.83 $x10^8$/kg. The results cannot be considered accurate as only fourteen percent of the patients had counts (Figure 8 Page 44).

Forty five percent of the patients had **MNC** counts, greater than 6.'2 $x10^8$/kg; forty one percent of them achieved a "12 month post transplant trilineage response". Seventy three percent of the patients produced an early **RMI** response, only thirty one percent of them achieved a "12 month post transplant trlineage response". Sixty eight percent of the patients produced an early **ANC** Response, only thirty three percent achieved a "12 month post transplant trilineage response". Sixty eight percent of the patients produced an early **PLT** response; forty seven percent of them achieved a "12 month post transplant

trilineage response". A higher than average **MNC** appeared to generate a greater than fifty percent early response in all three parameters, particularly in the **RMI** and **ANC**. The early **PLT** response did however appear to indicate a more positive projection on medium term success than the other two.

Eighty percent of the patients had **CFU-GM** counts, only thirty three percent of the patients generated counts greater than the mean of 26.10 $x10^4$/kg, and thirty three percent of them achieved a "12 month post transplant trilineage response". Sixty seven percent of the patients produced an early **RMI** response, only twenty five percent achieved a "12 month post transplant trilineage response". Sixty seven percent of the patients produced an early **ANC** response; thirty eight percent achieved a "12 month post transplant trilineage response". Seventy five percent of the patients produced an early **PLT** response; thirty three percent of them achieved a "12 month post transplant trilineage response". A higher than average **CFU-GM** count generated early responses in all three parameters in more than fifty percent of the patients, the highest being the **PLT**, indicating that the **PLT** was the most responsive parameter. Less than fifty percent of the patients with early responses achieved a "12 month post transplant trilineage response", specifying a less positive projection on medium term success.

Only fourteen percent of the patients had **CD34+** counts, three of them achieving a "12 month post transplant trilineage response". Only two of the patients produced counts greater than the mean, one died. Due to the low numbers and fluctuation within the counts no correlation could be established between higher **CD34+** counts and early responses. The numbers were too low for accurate evaluation.

4.2.6. Response Range for Transplant Patients

The ninety two percent successful engraftments were used to establish a **RMI**, **ANC**, and **PLT** Response Range. The patient's daily mean was calculated for the first forty days, three months, four months, five months and six months post transplant, a **2SD** was established for each parameter (Figure 9 Page 45).

4.2.6.1. Reticulocyte Maturation Index (RMI)

Twelve percent of the patients generated **RMI** values greater than **2SD** at least once within the first forty days. One of these patient achieved a "12 month post transplant trilineage response", four died and two relapsed. Ten percent generated **RMI** values below the **2SD** at least once during the first forty days, three patients resulted in "graft failure", one died within thirty days, one relapsed and two achieved a "12 month post transplant trilineage response". **RMI** results, above or below **2SD** within the first forty days displayed no indication of expected outcome for the transplants.

4.2.6.2. Absolute Neutrophil Count (ANC)

Eighteen percent of the patients examined, generated **ANC** values greater than **2SD** at least once during the first forty days. Eight patients achieved a "12 month post transplant trilineage response", one died from unrelated transplant causes, although he was doing well until this point. Thirty nine percent generated an **ANC** of zero at some point. **ANC** counts above **2SD** within the first forty days, with no clinical infection, appeared to reveal an excellent chance at achieving a "12 month post transplant trilineage response".

4.2.6.3. Platelet Count (PLT)

At one point most patients achieved a **PLT** greater than **2SD**. No patients produced **PLT** of zero within the first forty days, although counts did fall below the mean at some point. Like the **RMI** the **PLT** values appeared to exhibit little effect on transplant outcome.

4.2.7. "Day 14" Bone Marrow Biopsy on Transplant Patients

Only thirty nine percent of the patients received "day 14" bone marrow biopsies. The two **SAA** allo**BMT** patients had biopsies performed following the first and second transplant. All the patients that indicted a trilineage response on "day 14" produced a trilineage response on "day 28". Cases worth mentioning are:- An **AML** auto**PBSCT**, and two **SAA** allo**BMT** "graft failure" patients revealed an aplastic picture on the "day 14" marrow biopsy, and "day 28". Although aplasia was seen early, they all died. An **AML** auto**PBSCT** patient with delayed **ANC** and **PLT PB** results, did however have trilineage responses on both the "day 14" and "day 28" marrow biopsies. These four cases indicate the importance of monitoring **PB** counts parallel with biopsy analysis.

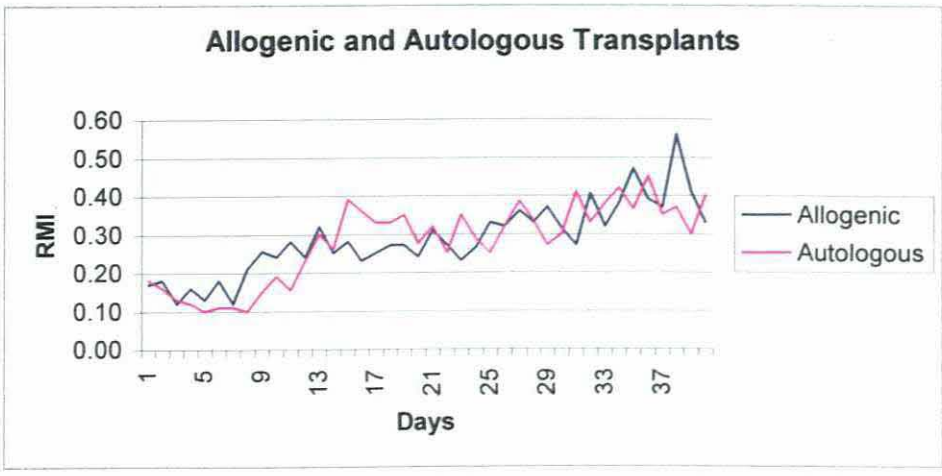
4.3. Comparison of Allogenic and Autologous Transplants

Thirty two allogenic and thirteen autologous transplants were analysed.

4.3.1. The Reticulocyte Maturation Index (RMI) Response

4.3.1.1. The Mean and Median Response Time for the Reticulocyte Maturation Index (RMI) between Allogenic and Autologous Transplant Patients

The mean Response Time for the allogenic and autologous transplants was nine days, with a range of one to seventeen days (Figure 5 Page 39). The median Response Time for the allogenic transplants was nine days and the autologous transplants ten days, both with a range of one to seventeen days (Figure 6 Page 40). All the patients responded within seventeen days.



Graph 7: The mean **RMI** Response Time, in days, for thirty two allogenic and thirteen autologous transplant patients.

The allogenic transplant mean **RMI** of 0.37 IU was seen within forty days (Graph 7 Page 60), at three months 0.24 IU, at four months 0.23 IU, at five months 0.21 IU and at six months 0.26 IU, a forty two percent mean decrease (Figure 10 Page 46). A median **RMI** of 0.33 IU was seen within forty days, 0.21 IU at three months, 0.26 IU at four months,

0.20 IU at five months and 0.26 IU at six months, a twenty seven percent median decrease (Figure 11 Page 46).

An autologous transplant mean **RMI** of 0.41 IU was seen within forty days (Graph 7 Page 60), 0.30 IU at three months, 0.31 IU at four months, 0.21 IU at five months and 0.16 IU at six months, a one hundred and fifty six percent mean decrease (Figure 10 Page 46). A median of 0.40 IU was seen within forty days, 0.33 IU at three months, 0.32 IU at four months, 0.21 IU at five months and 0.16 IU at six months, a one hundred and one hundred and fifty percent median decrease (Figure 11 Page 46).

Although the **RMI** mean Response Time was the same for both the allogenic and autologous transplants, a considerable decrease in mean counts from forty days to six months was indicated (from 0.37 IU to 0.26 IU), that is forty two percent for the allogenic transplants. The autologous group **RMI** decreased from 0.41 to 0.16 IU, one hundred and fifty six percent. The greater decrease in values over the six month period for autologous transplants leads us to believe that erythrocyte recovery medium term is far less stable in autologous transplants than allogenic transplants.

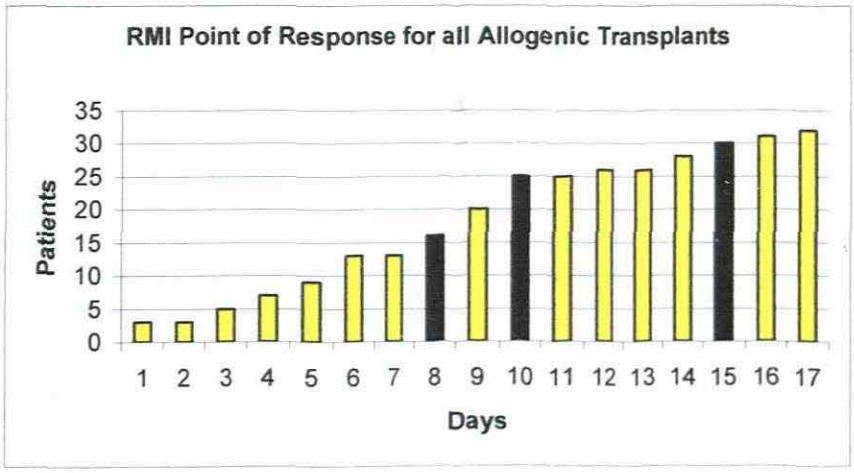
As a result the autologous transplants produced a percentage decrease double that of the allogenic transplants within six months. Perhaps the allogenic **RMI** response was healthier due to higher patient numbers analysed, or perhaps they were superior responders, possibly due to improved progenitor harvest quality. Only a thirty four percent of the allogenic transplants achieved a "12 month post transplant trilineage response" compared to seventy one percent in the autologous transplants.

4.3.1.2. The Point of Response for the Reticulocyte Maturation Index (RMI) between Allogenic and Autologous Transplant Patients

Even with large number differences between the thirty two allogenic and thirteen autologous transplants, the **RMI** Point of Response for the two was considerably close.

- **Allogenic Transplants**

A 95% Point of Response was seen within fifteen days, 50% within eight days and 75% within ten days (Graph 8 Page 62).

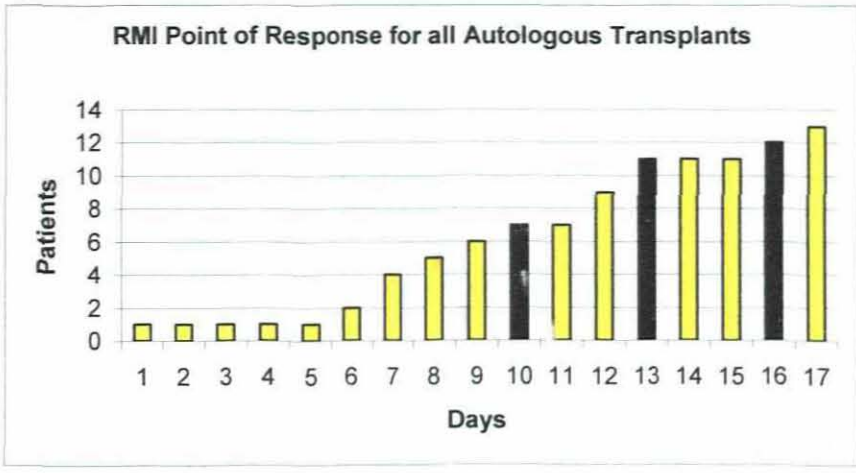


Graph 8: The **RMI** Point of Response, in days, for thirty two allogenic transplant patients, 50%, 75% and 95% are highlighted.

Ninety four percent of the patients fell within 95% Point of Response. Two patients responded on day sixteen and seventeen respectively, a **CML** allo**PBSCT** and an **AML** allo**PBSCT**, neither were considered "delayed responses".

- **Autologous Transplants**

A 95% Point of Response was seen within sixteen days, 50% within ten days and 75% within thirteen days (Graph 9 Page 63).



Graph 9: The **RMI** Point of Response, in days, for thirteen autologous transplant patients, 50%, 75% and 95% are highlighted.

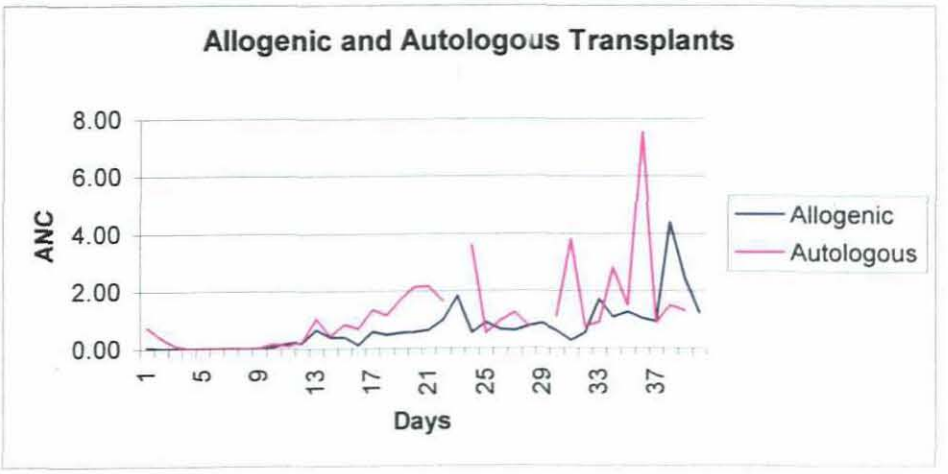
Ninety two percent of the patients responded within 95% Point of Response. One patient a **NHL** auto**PBSCT** responded on day seventeen, and was not considered a "delayed response".

The allogenic transplants produced a **RMI** Point of Response one day earlier than the autologous transplants. This was possibly of no significance.

4.3.2. The Absolute Neutrophil Count (ANC) Response

4.3.2.1. The Mean and Median Response Time for the Absolute Neutrophil Count (ANC) between Allogenic and Autologous Transplant Patients

The mean Response Time for the allogenic transplants was sixteen days, with a range of six to thirty two days, and fifteen days for the autologous transplants with a range with a range of nine to twenty two days (Figure 5 Page 39). The median Response Time was thirteen days for the allogenic transplants, with a range of six to thirty two days and fifteen with a range of nine to twenty two days (Figure 6 Page 40).



Graph 10: The mean **ANC** Response Time, in days, for thirty two allogenic and thirteen autologous transplant patients.

An allogenic transplant mean **ANC** of 1.63 $x10^9$/l was seen within forty days (Graph 10 Page 64), 2.30 $x10^9$/l at three months, 2.05 $x10^9$/l at four months. 2.18 $x10^9$/l at five months and 2.64 $x10^9$/l at six months, a sixty two percent mean increase (Figure 12 Page 47). The median of 1.23 $x10^9$/l was seen within forty days, 2.44 $x10^9$/l at three months, 1.89 $x10^9$/l at four months, 1.78 $x10^9$/l at five months and 2.28 $x10^9$/l at six months, an eighty five percent median increase (Figure 13 Page 47).

An autologous transplant mean **ANC** of 1.12 $x10^9$/l was seen within forty days (Graph10 Page 64), 2.89 $x10^9$/l at three months, 3.20 $x10^9$/l at four months, 1.90 $x10^9$/l at five months and 3.03 $x10^9$/l at six months, a one hundred and seventy one percent mean increase (Figure 12 Page 47). The median was 1.30 $x10^9$/l at forty days, 1.54 $x10^9$/l at three months, 2.24 $x10^9$/l at four months, 1.78 $x10^9$/l at five months and 2.88 $x10^9$/l at six months, a one hundred and twenty two percent median increase (Figure 13 Page 47).

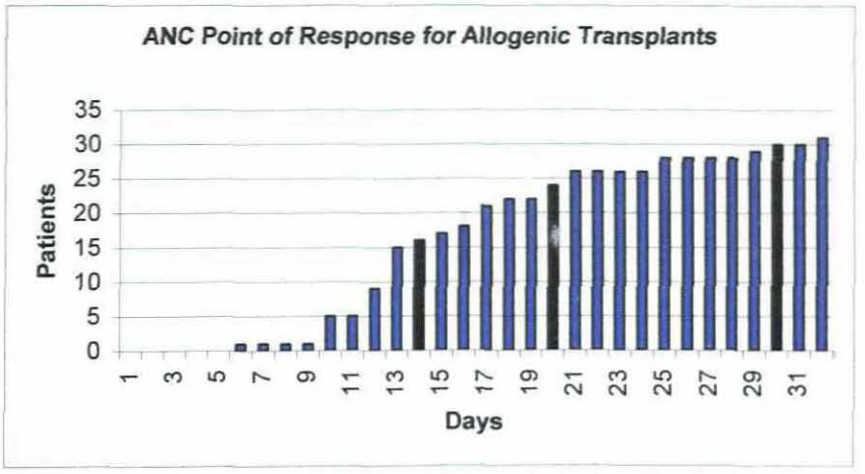
The mean **RMI** Response Time for the allogenic transplant group was seen eight days earlier than the **ANC** Response Time. There was only a six day difference between the two in the autologous transplant group. It may be significant, because within forty days the allogenic transplant group also generated a higher mean **ANC**, but a lower count and percentage increase than the autologous transplant group at six months.

Both the allogenic and autologous mean **ANC** increased over six months. There was a greater difference between the mean and the median in the autologous transplants particularly at three and four months, which indicated an unstable response earlier in the transplant. The autologous transplants had a far less stable mean **ANC** response within the first forty days (Graph 10 Page 64); they did however steady out within six months. This was consistent with the fact that the autologous transplants resulted in a higher percentage of patients achieving a "12 month post transplant trilineage response".

4.3.2.2. The Point of Response for the Absolute Neutrophil Count (ANC) between Allogenic and Autologous Transplant Patients

- **Allogenic Transplants**

A 95% Point of Response was seen within thirty days, 50% within fourteen days and 75% within twenty days (Graph 11 Page 66).



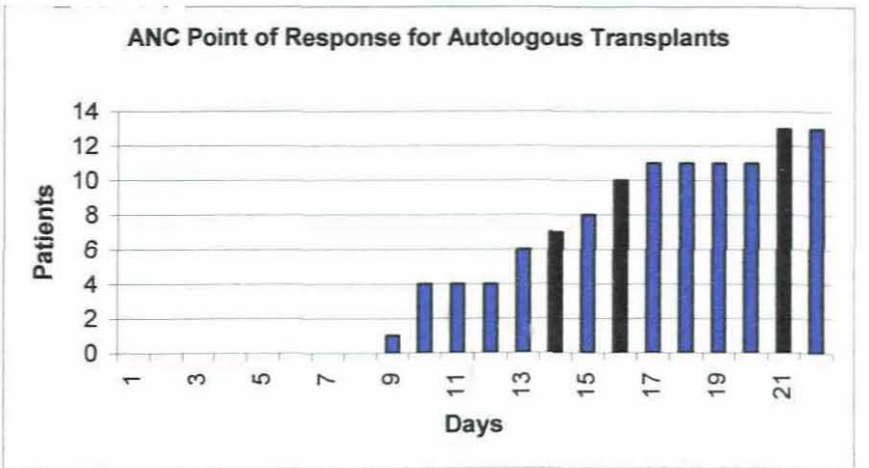
Graph 11: The **ANC** Point of Response, in days, for thirty two allogenic transplant patients, 50%, 75% and 95% are highlighted.

Ninety four percent of the patients fell within 95% Point of Response. Two patients did not, one a **NHL** allo**PBSCT** patient with a "slightly delayed response" at day thirty and a **CML** allo**PBSCT** patient with a "delayed response" at day fifty seven. An **AML** allo**PBSCT** who responded within thirty days fell within the 95% limit for this group, although she was considered "slightly delayed response" in the total transplant group.

The **RMI** Point of Response was fifteen days earlier than the **ANC** in the allogenic transplant group compared to eight days earlier mean Response Time, the indication being that the **RMI** parameter is as an excellent early engraftment tool.

- **Autologous Transplants**

A 95% Point of Response was seen within twenty one days, 50% within fourteen days and 75% within sixteen days (Graph 12 Page 67).



Graph 12: *The* **ANC** *Point of Response, in days for thirteen autologous transplant patients,* 50%, 75% and 95% are highlighted.

One hundred percent of the patients fell within 95% Point of Response.

The **RMI** Point of Response was five days earlier than the **ANC** in the autologous transplant group, although the mean Response Time was only one day earlier. The **RMI** is indicated as being a good engraftment tool.

The **ANC** Point of Response in the autologous transplant group was seen nine days earlier than the allogenic transplant group. Autologous transplants utilise the patient's own cells, we believe that these particular cells would have a higher affinity for recognition therefore resulting in an earlier and healthier response. This aspect of recognition we have termed a "pattern of recognition" encountered within the autologous transplant population. The autologous transplant group also produced a healthier **ANC** increase within six months. The **RMI** value for the autologous transplant group

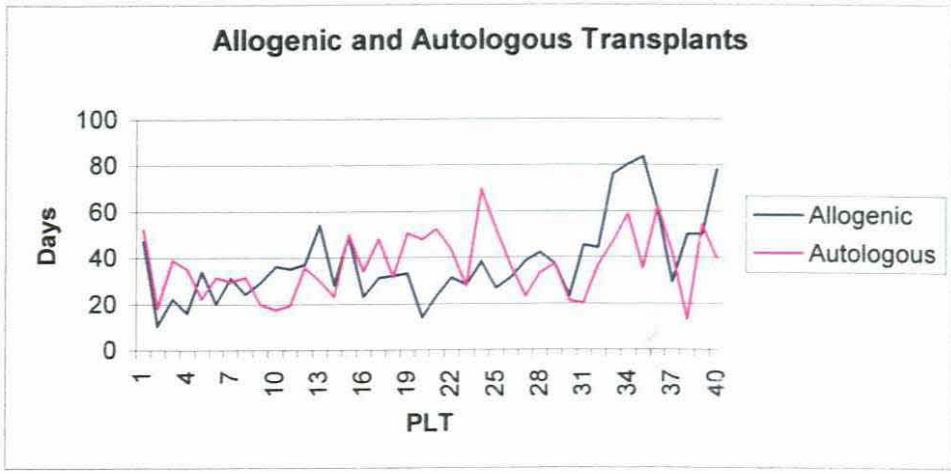
decreased by double that of the allogenic group, this was possibly a result of what appeared to be less aggressive preconditioning within the allogenic transplants.

The **RMI** Point of Response was fifteen days earlier than the **ANC** in the allogenic transplant group, and five days earlier in the autologous transplant group. The **RMI** parameter was an excellent early engraftment indicator, particularly regarding the "delayed response" patients. It allowed engraftment prediction without undue concern for slow **ANC** and **PLT** responses, examples were seen in existing patients.

4.3.3. The Platelet Count (PLT) Response

4.2.3.1. The Mean and Median Response Time for the Platelet Count (PLT) between Allogenic and Autologous Transplant Patients

The mean Response Time of sixteen days was seen in the allogenic transplants with a range of one to thirty six days. The mean Response Time for the autologous transplants was seventeen days, with a range of ten to twenty eight days (Figure 5 Page 39). A median Response Time of twelve days was seen in the allogenic transplants, with a range of one to thirty six days and fifteen days. The median Response Time in the autologous transplants was fifteen days, with a range of ten to twenty eight days (Figure 6 Page 40).



Graph 13: The mean **PLT** Response Time, in days, for thirty two allogenic and thirteen autologous transplant patients.

An allogenic transplant mean **PLT** of 58 $x10^9$/l was seen within forty days (Graph 13 Page 68), 116 $x10^9$/l at three months, 137 $x10^9$/l at four months, 140 $x10^9$/l at five months

and 150 $x10^9$/l at six months, a one hundred and fifty nine percent mean increase (Figure 14 Page 48). A median of 78 $x10^9$/l was seen within forty days (Graph 13 Page 68), 109 $x10^9$/l at three months, 141 $x10^9$/l at four months, 126 $x10^9$/l at five months and 161 $x10^9$/l at six months, a one hundred and six percent median increase (Figure 15 Page 48). An autologous transplant **PLT** mean of 40 $x10^9$/l was seen within forty days (Graph 13 Page 68), 64 $x10^9$/l at three months, 77 $x10^9$/l at four months, 92 $x10^9$/l at five months and 115 $x10^9$/l at six months, a one hundred and eighty eight percent mean increase (Figure 14 Page 48). A median of 50 $x10^9$/l was seen within forty days, 70 $x10^9$/l at three months, 72 $x10^9$/l at four months, 73 $x10^9$/l at five months and 107 $x10^9$/l at six months, a one hundred and fourteen percent median increase (Figure 15 Page 48).

The allogenic transplant group produced a mean **PLT** Response Time one day earlier than the autologous transplant group. No significant difference was detected.

The allogenic transplant group **RMI** mean Response Time was eight days prior to both the **ANC** and **PLT**. The **ANC** Response Time for autologous transplants was two days prior to the **PLT** and the **RMI** was eight days prior. Although the allogenic transplant group produced the same mean Response Time for the **ANC** and **PLT**, the mean **RMI** Response Time was still significantly faster, indicating **RMI** as a good prediction tool.

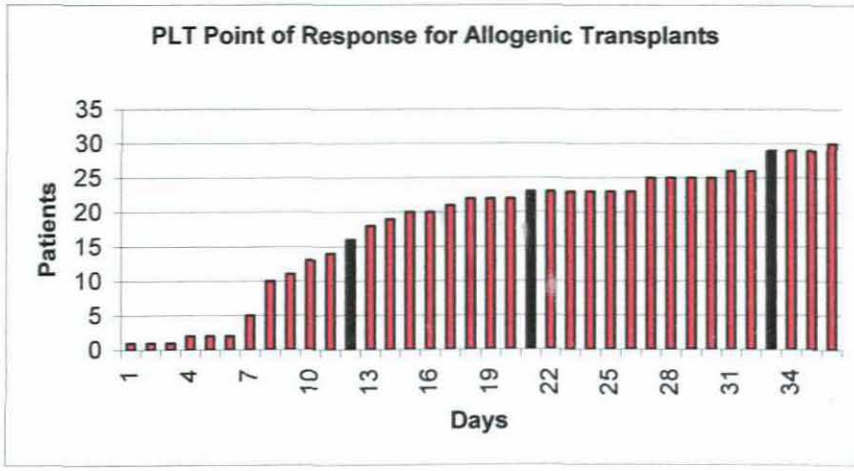
The autologous transplant group produced a significantly faster mean **RMI** Response Time, but slower mean **PLT** Response Time. The **PLT** generated the most significant percentage recovery within six months of all three parameters, at one hundred and eighty eight percent, possibly as a result of the excellent response seen in patients with greater than average **CFU-GM** counts.

The **PLT**, upon reaching the accepted value of 50 $x10^9$/l days fluctuated above and below the limit continuously throughout the forty day period, very rarely falling below 20 $x10^9$/l, in both groups (Graph 13 Page 68). The increase in **PLT** count was not as steady as the **ANC** and the **RMI**; it tended to remain at a level between 20 $x10^9$/l and 60 $x10^9$/l for six months.

4.3.3.2. The Point of Response for the Platelet Count (PLT) between Allogenic and Autologous Transplant Patients

- **Allogenic Transplants**

A 95% Point of Response was seen within thirty three days, 75% within twenty days and 50% within twelve days (Graph 14 Page 70).



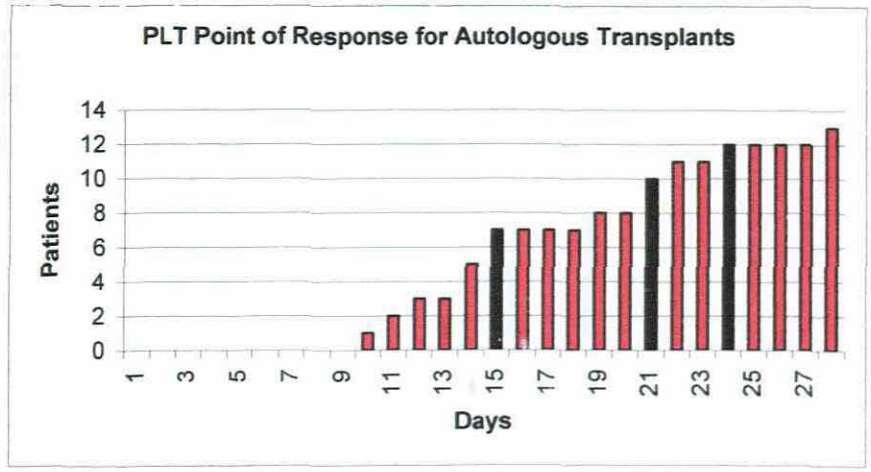
Graph 14: The **PLT** Point of Response, in days, for thirty allogenic transplant patients, 50%, 75% and 95% are highlighted.

Ninety one percent of the patients fell within 95% Point of Response. Three patients fell outside the allogenic transplant group limit, one **CML** allo**PBSCT** patient with a thirty six day "slightly delayed response" and two **CML** allo**BMT** patients with a "delayed response" at day seventy three and forty six.

The **RMI** Point of Response was seen twenty one days prior to the **PLT**, and **ANC** thirteen days prior. The **RMI** Point of Responses was seen significantly earlier than the **ANC** and **PLT.** This indicated the significance of early **RMI** engraftment, particularly regarding the "delayed response" patients and allowed the **RMI** as an indicator for engraftment without undue concern for slow **ANC** and **PLT** responses.

- **Autologous Transplants**

A 95% Point of Response was seen within twenty four days, 75% within twenty one days and 50% within fifteen days (Graph 15 Page 71).



Graph 15: The **PLT** Point of Response, in days, for twelve autologous transplant patients, 50%, 75% and 95% are highlighted.

Ninety two percent of the patients responded within 95% Point of Response. One patient, an **AML** auto**PBSCT**, responded on day twenty eight, and was considered a "slightly delayed response".

An **RMI** Point of Response was seen eight days prior to the **PLT** response and three days prior response by **ANC** to **PLT,** indicating an earlier erythrocyte response.

The autologous transplant group **ANC** and **PLT** Point of Response were nine days earlier than the allogenic transplants, indicating a "pattern of recognition" within both the **ANC** and **PLT**.

4.3.4. Preconditioning in Allogenic and Autologous Transplants

Thirty four allogenic transplants were preconditioned, including the two **SAA** allo**BMT** "graft failure" patients; the following preconditioning regimes were used:

- Twenty six **Cyclo/TBI/TNI.**
- One **Bu/Mel/Thiotep**.
- Three **Bu/Cy**.
- Four **Cyclo/TNI**.

There were fourteen autologous transplants including the **AML** auto**PBSCT** "graft failure":

- Three **Bu/Mel/Thiotep**.
- Six **Bu/Cy**.
- Five **Cyclo/TBI/TNI.**

Seventy one percent of the allogenic transplants received **Cyclo/TBI/TNI** preconditioning. Sixty percent of the patients produced an early **RMI** response; thirty three percent of them achieved a "12 month post transplant trilineage response". Sixty eight percent of the patients produced an early **ANC** response; twenty eight percent of them achieved a "12 month post transplant trilineage response". Sixty four percent of the patients produced an early **PLT** response, only thirty one percent of them achieved a "12 month post transplant trilineage response. More than fifty percent of the patients generated early responses in all three parameters; this indicated minimal stromal damage. All three had less than fifty percent achieving a "12 month post transplant trilineage response", indicating a less positive medium term prognosis.

Of the three allogenic patients that received **Bu/Cy** as preconditioning, two died, one of them produced an early **PLT** response, the third achieved a "12 month post transplant trilineage response", he too only produced an early **PLT** response. The numbers were too low for accurate evaluation. Of four patients that received **Cyclo/TNI** preconditioning, the two **SAA** allo**BMT** died, one with an early **RMI** and **PLT** response, the two **SAA** allo**PBSCT** achieved a "12 month post transplant trilineage response", one with early responses in all three parameters. The numbers were too low for accurate evaluation.

The only allogenic transplant to receive **Bu/Mel/Thiotep**, generated no early responses in any of the three peripheral blood parameters, she died.

Thirty six percent of the autologous transplants received **Cyclo/TBI/TNI** preconditioning, three of them (sixty percent) generated early responses in all three parameters and all of them achieved a "12 month post transplant trilineage response". One died, she generated no early responses. The fifth patient with only an early **ANC** response achieved a "12 month post transplant trilineage response".

Of the forty three percent who received **Bu/Cy** preconditioning, only one patient (seventeen percent) produced an early **RMI** response, he achieved a "12 month post transplant trilineage response". Two patients (thirty three percent) produced an early **ANC** response, one died. Two (thirty three percent) produced early **PLT** responses, both achieved a "12 month post transplant trilineage response". Of the three patients that received **Bu/Mel/Thiotep**, one generated early responses in all three parameters, she achieved a "12 month post transplant trilineage response", the second produced an early **PLT** response and the third an early response in the **ANC** and **PLT**, and they died. Even with low numbers in all the preconditioning regimes, more than fifty percent of the patients generated early responses in all three parameters. This indicated minimal stromal damage. More than fifty percent of them achieved a "12 month post transplant trilineage response", permitting a positive projection on medium term success.

4.3.5. Mononuclear Count (MNC), Colony Forming Unit – Granulocytic Monocytic (CFU-GM) and Cluster Designation CD34+ Counts (CD34+) in Allogenic and Autologous Transplants

MNC were performed on all the allogenic transplant patients, with a mean of 5.83 $x10^8$/kg. Eighty five percent of the patients had **CFU-GM** performed, with a mean of 26.9 $x10^4$/kg; nine percent were contaminated with bacteria and six percent with no growth. Seventy two percent of the patients had **CD34+** counts, generating a mean of 62.35. **MNC** were performed on all the autologous transplant patients, with a mean of 6.53 $x10^8$/kg. Only sixty two percent of the patients had **CFU–GM** performed, with a mean of 22.15 $x10^4$/kg, thirty one percent with no growth and seven percent were

contaminated. Only twenty nine percent of the patients had **CD34+** counts with a mean of 137.55 (Figure 8 Page 43).

Seventy one percent of the allogenic transplants generated **MNC** greater than the mean, fifty percent of the patients produced an early **RMI** response while only thirty percent of them achieved a "12 month post transplant trilineage response". Sixty percent of the patients produced early **ANC** and **PLT** responses, only twenty five percent of each achieved a "12 month post transplant trilineage response". Because more than fifty percent of the patients generated early responses in all three parameters, this indicated those allogenic transplants with **MNC** greater than average produced earlier Response Times. Unfortunately because less than fifty percent of the patients achieved a "12 month post transplant trilineage response", this indicated a less positive projection for medium term success.

Thirty six percent of the autologous transplants generated **MNC** greater than the mean. Sixty seven percent of the patients produced an early **RMI** response, and seventy five percent of them achieved a "12 month post transplant trilineage response". Fifty percent of the patients produced an early **ANC** response; one hundred percent achieved a "12 month post transplant trilineage response". Sixty six percent of the patients produced an early **PLT** response; one hundred percent achieved a "12 month post transplant trilineage response". Although the percentage of early responses was greater than fifty percent in all three parameters, they were far higher than the allogenic transplants; this indicated healthier responses in autologous transplants with higher **MNC**. The medium term projection was perfect with one hundred percent "12 month post transplant trilineage response".

Of the eighty percent allogenic transplants that had **CFU-GM** counts, only thirty nine percent produced counts greater than the mean. Sixty four percent of the patients produced an early **RMI** response, only thirty three percent of them achieved a "12 month post transplant trilineage response". Sixty four percent of the patients produced an early **ANC** response; fifty percent of them achieved a "12 month post transplant trilineage

response". Fifty percent of the patients produced an early **PLT** response, only twenty five percent achieved a "12 month post transplant trilineage response". More than fifty percent of the patients generated early responses particularly in the **RMI** and **ANC**; this indicated those patients with **CFU-GM** counts greater than the mean resulted in earlier parameter response. Unfortunately less than fifty percent completed a positive projected medium term success.

Of the fifty seven percent autologous transplants that had **CFU-GM** counts, only twenty five percent (two patients) generated counts greater than the mean. One patient produced an early **RMI** response, and achieved a "12 month post transplant trilineage response". Two patients generated early **ANC** and **PLT** responses; one of each achieved a "12 month post transplant trilineage response". Unfortunately the patient numbers were too low for accurate evaluation.

Five of the seven patients with **CD34+** counts were allogenic transplants, three of them with counts greater than the mean. Two patients generated early responses in all three parameters, one died. The third patient produced early **ANC** and **PLT** responses, she died. The numbers were too low for accurate evaluation. Only two patients had **CD34+** counts in the autologous transplants, both with extensively different results, although both with early responses and both achieving a "12 month post transplant trilineage response". Although patient numbers were low, autologous transplants with an early parameter response, **MNC** counts above 6 x10^8/kg, and **CD34+** counts above 27.00 were considered good candidates for successful transplant.

4.3.6. Response Range for Allogenic and Autologous Transplant Patients

4.3.6.1. Reticulocyte Maturation Index (RMI)

Of the patients that generated **RMI** values greater than **2SD**, eighty three percent (five patients) of them were allogenic transplants. Only seventeen percent (one patient) were autologous. The autologous transplant was the only one to achieve a "12 month post transplant trilineage response". This was possibly of no significance. The five (seventeen percent) patients with **RMI** values below **2SD** were all allogenic transplants.

Only one achieved a "12 month post transplant trilineage response", this was possibly of no significance. Only one patient, an autologous transplant, generated a **RMI** count above **2SD** and achieved a "12 month post transplant trilineage response", unfortunately this was possibly of no significance.

4.3.6.2. Absolute Neutrophil Count (ANC)

Of the nine patients whose **ANC** rose above **2SD**, twenty two percent (two) were allogenic transplants and seventy seven (seven) were autologous transplants. Both the allogenic and eighty six percent of autologous transplants achieved a "12 month post transplant trilineage response".

4.3.6.3. Platelet Count (PLT)

The **PLT** revealed no information; all patients achieved counts above and below **2SD** within the first forty days.

4.3.7. "Day 14" Marrow Biopsy Results on Allogenic and Autologous Transplant Patients

Forty three percent of the allogenic transplants and eight percent of the autologous transplants received "day 14" marrow biopsies. These biopsies revealed no additional information compared to the "day 28" marrow biopsies.

One patient is worth mentioning, an **AML** allo**PBSCT**, who received only a "day 28" marrow biopsy, indicating a low erythrocyte and megakaryocyte response. The **PB** counts revealed an **RMI** response on day one; **ANC** and **PLT** responses were both day eight, the **PLT** then decreased. Had she received a "day 14" marrow biopsy the lack of megakaryocyte engraftment may have been detected earlier, along with the **PB** counts. This example indicates the importance of **PB** counts, in conjunction with histology following transplant. In the case of the two **SAA** allo**BMT** "graft failures", "day 14" marrow biopsies were done, both revealed lack of engraftment, along with the **PB** counts. None of the **SAA** allo**PBSCT** received "day 14" marrow biopsies, although one revealed a "delayed response" in all three **PB** parameters.

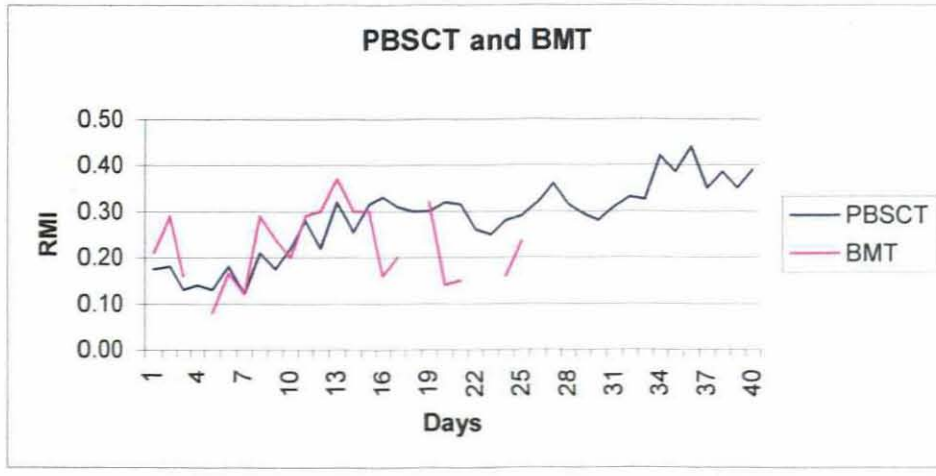
4.4. Comparison of Peripheral Blood Stem Cell Transplant (PBSCT) and Bone Marrow Transplant (BMT) Patients

There were thirty five **PBSCT** and ten **BMT**.

4.4.1. The Reticulocyte Maturation Index (RMI) Response

4.4.1.1. The Mean and Median Response Time for the Reticulocyte Maturation Index (RMI) between Peripheral Blood Stem Cell Transplant (PBSCT) and Bone Marrow Transplant (BMT) Patients

The **BMT** and produced a mean Response Time of eight days, with a range of one to fifteen days. The **PBSCT** produced a mean Response Time of nine days, with a range of one to seventeen days (Figure 5 Page 39). The **BMT** and **PBSCT** both had a median Response Time of nine days, with a range of one to fifteen days for **BMT** and one to seventeen days for the **PBSCT** (Figure 6 Page 40). All the patients responded within seventeen days.



Graph 16: The mean **RMI** Response Time, in days, for thirty five **PBSCT** and ten **BMT** patients.

A **PBSCT** mean **RMI** of 0.38 IU was seen within forty days (Graph 16 Page 77), 0.27 IU at three months, 0.26 IU at four months, 0.20 IU at five months and 0.19 IU at six months, a mean decrease of one hundred percent (Figure 10 Page 46). A median of 0.39 IU was seen within forty days, 0.27 IU at three months, 0.26 IU at four months, 0.20 IU at five months and 0.18 IU at six months, a median decrease of one hundred and seventeen percent (Figure 11 Page 46).

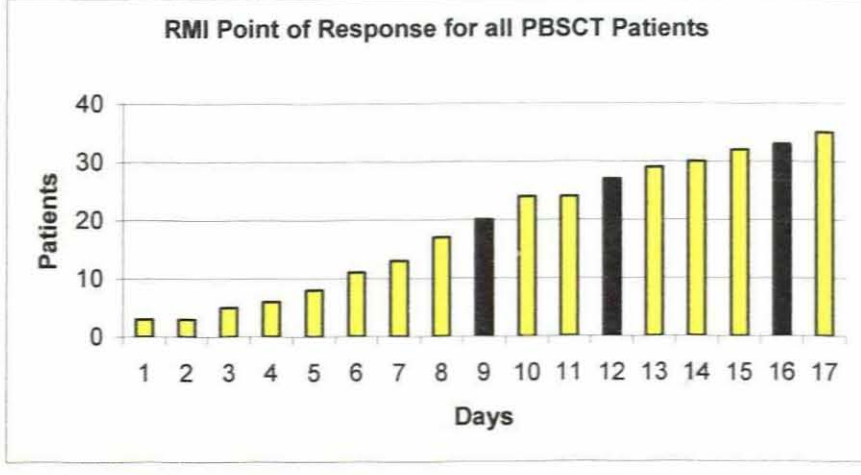
A **BMT** mean **RMI** of 0.45 IU was seen within forty days (Graph 16 Page 77), 0.25 IU at three months, 0.27 IU at four months, 0.23 IU at five months and 0.33 IU at six months, a mean decrease of thirty six percent (Figure 10 Page 46). A median of 0.45 IU was seen within forty days, 0.25 IU at three months, 0.31 IU at four months 0.23 IU at five months and 0.36 IU at six months, a median decrease of twenty five percent (Figure 11 Page 46).

The **PBSCT** produced a mean decrease from 0.38 IU to 0.19IU, one hundred percent within six months. The **BMT** produced the lowest mean decrease of 0.45 IU to 0.33IU, thirty six percent within six months. The **BMT** produced the highest **RMI** count at forty days, which indicated not only a more stable erythrocyte response within forty days but also over a six month period.

4.4.1.2. The Point of Response for the Reticulocyte Maturation Index (RMI) between Peripheral Blood Stem Cell Transplant (PBSCT) and Bone Marrow Transplant (BMT) Patients

- **PBSCT**

A 95% Point of Response was seen within sixteen days, 75% within twelve days and 50% within nine days (Graph 17 Page 78).

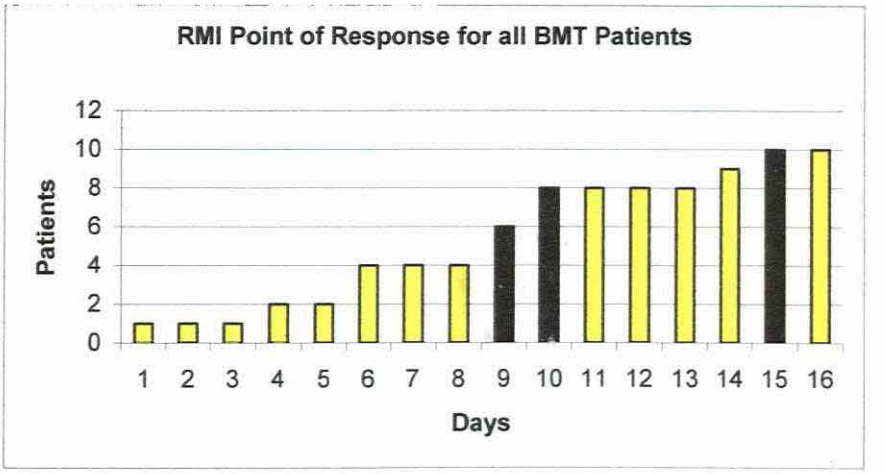


Graph 17: The **RMI** Point of Response, in days for thirty five **PBSCT** patients, 50%, 75% and 95% are highlighted.

Ninety four percent of the patients fell within 95% Point of Response. Two patients an **AML** allo**PBSCT** and a **CML** allo**PBSCT** produced **RMI** responses within seventeen days neither were considered a "delayed response".

- **BMT**

A 95% Point of Response was see within fifteen days, 75% within ten days and 50% within nine days (Graph 18 Page 79).



Graph 18: The **RMI** Point of Response for all the **BMT** patients, 10 in total, 95%, 75% and 50% are highlighted, all 10 patients fell within the 95% limit.

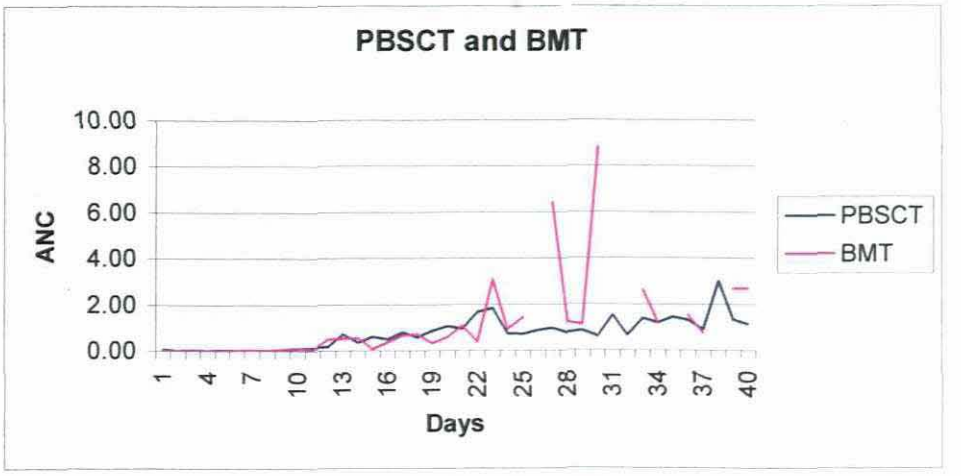
One hundred percent of the patients responded within 95% Point of Response.

The **PBSCT RMI** Point of Response was only one day earlier than the **BMT**; this was possibly of no significance.

4.4.2. The Absolute Neutrophil Count (ANC) Response

4.4.2.1. The Mean and Median Response Time for the Absolute Neutrophil Count (ANC) between Peripheral Blood Stem Cell Transplant (PBSCT) and Bone Marrow Transplant (BMT) Patients

The **BMT** mean Response Time was seventeen days, with a range of six to thirty two days. The **PBSCT** mean Response Time was fifteen days, with a range of eight to thirty days (Figure 5 Page 39). The **BMT** and the **PBSCT** both produced a median Response Time of fifteen days, with a **BMT** range of six to thirty two days and the **PBSCT** eight to thirty days (Figure 6 Page 40). All the **BMT** patients responded within thirty two days and the **PBSCT** patients within thirty days.



Graph 19: The mean **ANC** Response Time, in days, for thirty five **PBSCT** and ten **BMT** patients.

A **PBSCT** mean **ANC** of 1.13 $x10^9$/l was seen within forty days (Graph 19 Page 80), 2.60 $x10^9$/l at three months, 2.57 $x10^9$/l at four months, 2.06 $x10^9$/l five months and 2.82 $x10^9$/l at six months, a mean increase of one hundred and fifty percent (Figure 12 Page 47). A median of 1.13 $x10^9$/l was seen within forty days, 2.25 $x10^9$/l at three months, 2.03 $x10^9$/l at four months, 1.79 $x10^9$/l at five months and 2.61 $x10^9$/l at six months, a median increase of one hundred and thirty one percent (Figure 13 Page 47).

A **BMT** mean **ANC** of 2.65 $x10^9$/l was seen within forty days (Graph 19 Page 80), 2.20 $x10^9$/l at three months, 2.01 $x10^9$/l at four months, 2.15 $x10^9$/l at five months and 2.64 $x10^9$/l at six months, no mean percentage difference was seen (Figure 12 Page 47). A median of 2.63 $x10^9$/l was seen within forty days, 1.53 $x10^9$/l at three months, 1.87 $x10^9$/l at four months, 2.09 $x10^9$/l at five months and 2.28 $x10^9$/l at six months, a median fifteen percent decrease (Figure 13 Page 47).

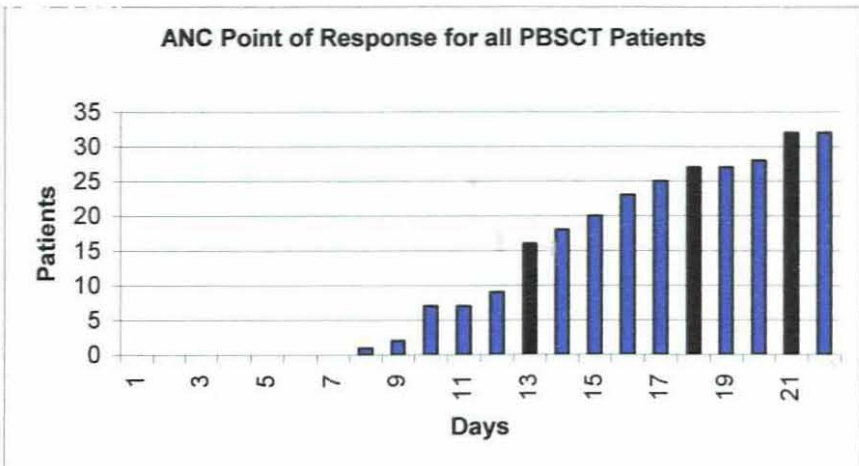
The mean **RMI** Response Time was nine days earlier than the **ANC** in the **BMT**, and six days earlier in the **PBSCT**, indicating an earlier erythrocyte recovery.

The **PBSCT ANC** mean Response Time improved steadily over six months by one hundred and fifty percent. The **BMT** was the only group with no increase over six months, at zero percent. The **BMT ANC** at forty days is significantly higher than the **PBSCT**, but the recovery of the **PBSCT** within six months brought the two within amicable difference of each other. The minimal drop in the **BMT ANC** was probably not as significant as the fact that there was no increase at all. The **BMT** only generated a thirty three percent and the **PBSCT** a forty nine percent "12 month post transplant trilineage response". The low percentage medium term recoveries were possibly due to small amount of stromal damage caused by the preconditioning, or poor early response by all three parameters in the **MNC** and **CFU-GM** seen in the **BMT.**

4.4.2.2. The Point of Response for the Absolute Neutrophil Count (ANC) between Peripheral Blood Stem Cell Transplant (PBSCT) and Bone Marrow Transplant (BMT) Patients

- **PBSCT**

A 95% Point of Response was seen within twenty one days, 75% within eighteen days and 50% within thirteen days (Graph 20 Page 82).



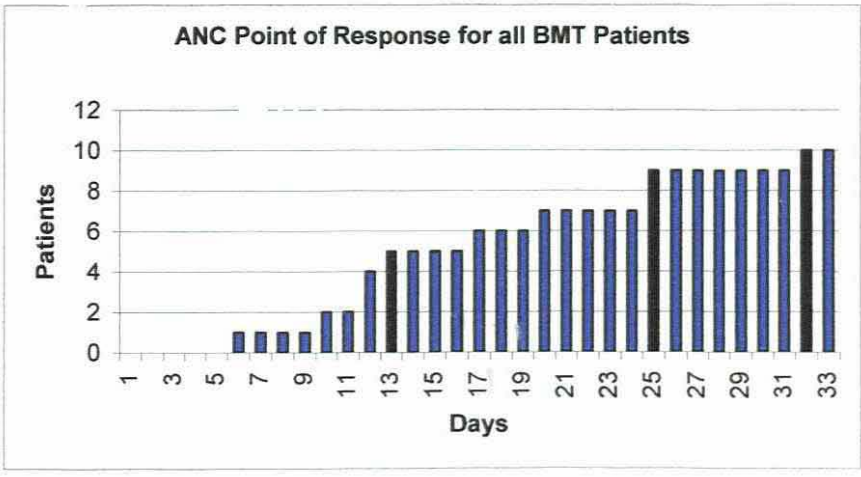
Graph 20: The **ANC** Point of Response, in days, for thirty two **PBSCT** patients, 50%, 75% and 95% are highlighted.

Ninety four percent of the patients fell within 95% Point of Response. Two patients a **CML** allo**PBSCT**, who responded on day twenty nine, and an **AML** allo**PBSCT**, who responded on day thirty, were considered "slightly delayed responses". One patient a **CML** allo**PBSCT**, who responded on day fifty seven, who was considered a "delayed response", was not used in the calculation. She achieved a "12 month post transplant trilineage response".

The **ANC** Point of Response was seen five days following the **RMI** response, indicating an earlier erythroid recovery.

- **BMT**

A 95% Point of Response was seen within thirty two days, 75% at twenty five days and 50% at thirteen days (Graph 21 Page 83).



Graph 21: The **ANC** Point of Response, in days, for ten **BMT** patients, 50%, 75% and 95% are highlighted.

One hundred percent of the patients fell within 95% Point of Response.

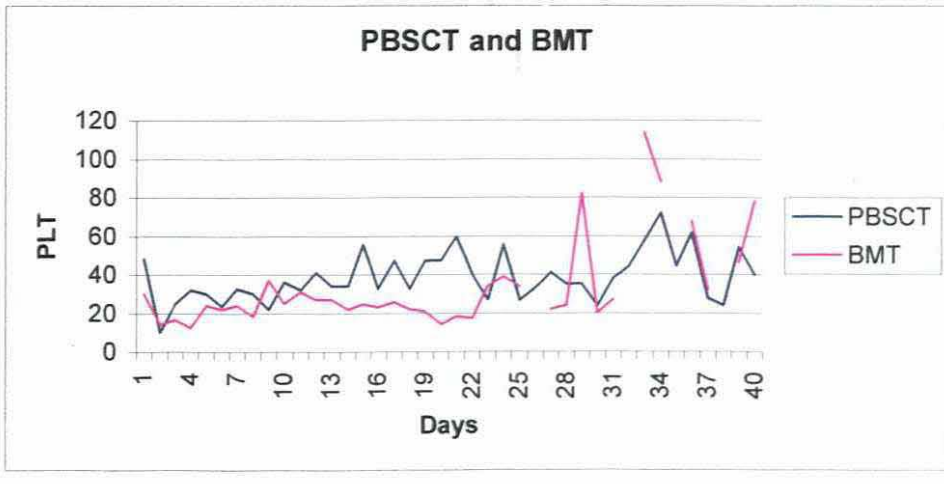
The **RMI** Point of Response was seen seventeen days earlier than the **ANC** in the **BMT**, but only four days earlier in the **PBSCT**. The **PBSCT** did generate earlier responses in all three parameters, possibly due to less aggressive preconditioning and higher **MNC** and **CFU-GM** counts.

The **PBSCT ANC** Point of Response was eleven days prior to the **BMT** response, and the **BMT RMI** response was seventeen days earlier than the **BMT ANC**. Even though the erythrocytic response was the same in both **PBSCT** and **BMT**, there was a preferred recovery in the granulocytic population in the **BMT** at forty days, possibly due to a higher number of functional pluripotent stem cells. The **PBSCT** patients did produce a healthier percentage increase of results within six months even though the count difference was minor.

4.4.3. The Platelet Count (PLT) Response

4.4.3.1. The Mean and Median Response Time for the Platelet Count (PLT) between Peripheral Blood Stem Cell Transplant (PBSCT) and Bone Marrow Transplant (BMT) Patients

The **BMT** mean Response Time was seventeen days, with a range of one to thirty three days. The **PBSCT** mean Response Time was sixteen days, with a range of seven to thirty six days (Figure 5 Page 39). The median Response Time for **BMT** was fifteen days, with a range of one to thirty three days. The **PBSCT** median Response Time was fourteen days, with a range of seven to thirty six days (Figure 6 Page 40). All **PBSCT** patients responded within thirty six days and eighty percent of the **BMT** responded within thirty three days.



Graph 22: The mean **PLT** Response Time, in days, for thirty five **PBSCT** and ten **BMT**.

A **PBSCT** mean **PLT** of 44 $x10^9$/l was seen within forty days (Graph 22 Page 84), 91 $x10^9$/l at three months, 115 $x10^9$/l at four months, 120 $x10^9$/l at five months and 137 $x10^9$/l at six months, a mean increase of seventy six percent (Figure 14 Page 48). A median **PLT** of 40 $x10^9$/l was seen within forty days, 78 $x10^9$/l at three months, 96 $x10^9$/l at four months, 119 $x10^9$/l at five months and 127 $x10^9$/l at six months, a median increase of sixty nine percent (Figure 15 Page 48).

A **BMT** mean **PLT** of 78 $x10^9$/l was seen within forty days (Graph 22 Page 84), 116 $x10^9$/l at three months, 120 $x10^9$/l at four months, 133 $x10^9$/l at five months and 137 $x10^9$/l at six months, a mean increase of two hundred and eleven percent (Figure 14 Page

48). A median **PLT** of 78 $x10^9$/l was seen within forty days forty days, 109 $x10^9$/l at three months, 112 $x10^9$/l at four months, 120 $x10^9$/l at five months and 132 $x10^9$/l at six months, a median increase of two hundred and eighteen percent (Figure 15 Page 48).

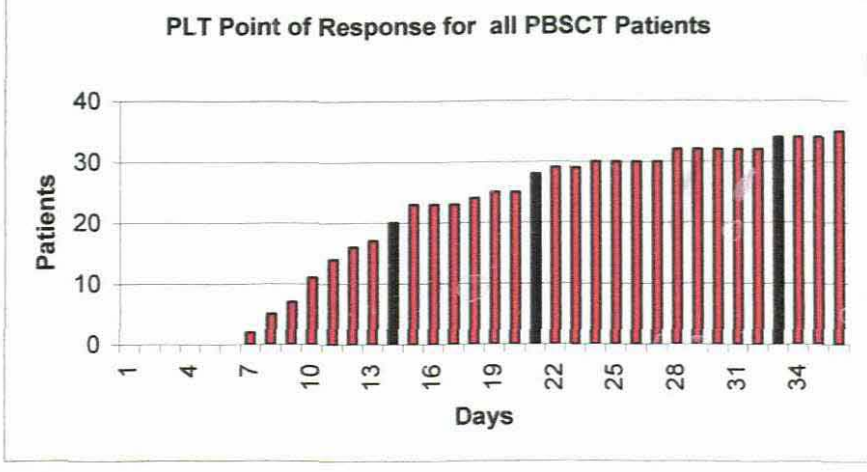
The **RMI** mean Response Time for both the **BMT** and the **PBSCT** was the earliest of all three parameters, indicating earlier erythroid recovery. The **ANC** and **PLT** mean Response Times were the same for both the **BMT** and the **PBSCT**.

Graphically the **PBSCT PLT** appeared steadier over forty days (Graph 22 Page 84), and was the least effected post transplant of all the parameters for both the **BMT** and the **PBSCT**. Even though the mean Response Time for **PBSCT** was six days earlier, the **BMT** produced a healthier mean count at forty days, almost double the **PBSCT.** This was possibly due to a superior number of pluripotent stem cells in **PBSCT**. In both transplant groups 95% of the patients responded within thirty three days, they had the same count at six months.

4.4.3.2. The Point of Response for the Platelet Count (PLT) between Peripheral Blood Stem Cell Transplant (PBSCT) and Bone Marrow Transplant (BMT) patients

- **PBSCT**

A 95% Point of Response was seen within thirty three days, 75% within twenty one days and 50% within fourteen days (Graph 23 Page 85).



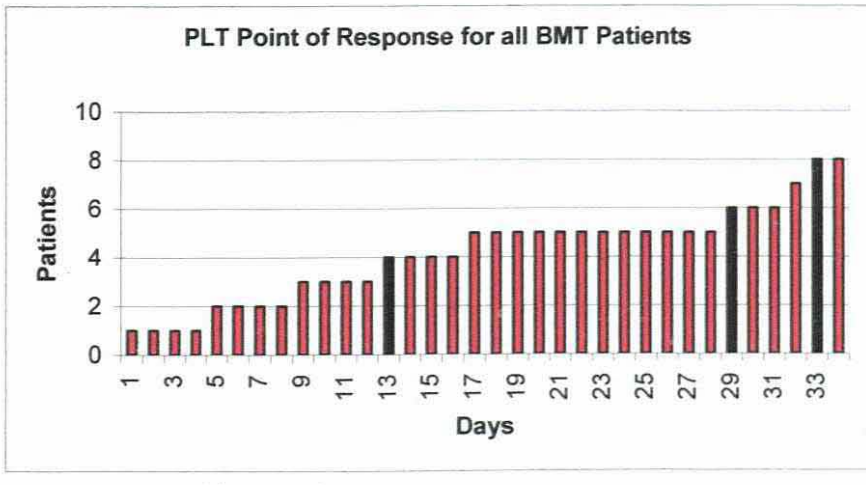
Graph 23: The **PLT** Point of Response, in days, for thirty five **PBSCT** patients, 50%, 75% and 95% are highlighted.

Ninety seven percent of the patients fell within 95% Point of Response. One patient a **CML** allo**PBSCT** responded on day thirty six, she was considered a "slightly delayed response".

The **PLT** Point of Response for the **PBSCT** was thirty three days. The **RMI** response was seventeen days earlier than the **PLT**, and the **ANC** response was twelve days prior to the **PLT**. Erythroid recovery was the earliest.

- **BMT**

A 95% Point of Response was seen within thirty three days, 75% in twenty nine days and 50% within thirteen days (Graph 24 Page 86).



Graph 24: The **PLT** Point of Response, in days, for eight **BMT** patients, 50%, 75% and 95% are highlighted.

Eighty percent of the patients responded within 95% Point of Response. Two **CML** allo**BMT** patients responded on days forty six and seventy three, both were considered "delayed responses".

The **RMI** Point of Response was eighteen days earlier than the **PLT,** the **ANC** response only one day earlier. Although there was very little difference between the **BMT** and **PBSCT PLT** response, the **ANC** response was seen earlier in the **PBSCT** than in the

BMT, and more significantly by eleven days, possibly due to richer **MNC** and **CFU-GM** counts.

All the "delayed responses" were **CML** transplants; the **CML** transplants produced the slowest responses of all the groups.

4.4.4. Preconditioning in Peripheral Blood Stem Cell Transplant (PBSCT) and Bone Marrow Transplant (BMT) Patients

Thirty seven **PBSCT** patients were preconditioned, including the **AML** auto**PBSCT** "failed response". The treatment regimes were as follows:

- Twenty one **Cyclo/TBI/TNI.**
- Nine **Bu/Cy.**
- Four **Bu/Mel/Thiotep.**
- Two **Cyclo/TNI.**
- One **Cyclo/Alg.**

Twelve **BMT** patients were preconditioned, including the two **SAA BMT** "failed responses". The treatment regimes were as follows:

- Ten **Cyclo/TBI/TNI.**
- Two **SAA** had **Cyclo/TNI.**

Fifty seven percent of the **PBSCT** patients received **Cyclo/TBI/TNI** preconditioning. Seventy six percent of the patients produced an early **RMI** response; forty four percent of them achieved a "12 month post transplant tri lineage response". Seventy one percent of the patients produced an early **ANC** and **PLT** response, only forty seven percent of them achieved a "12 month post transplant trilineage response". Early **RMI**, **ANC** and **PLT** responses were seen in more than fifty percent of the patients, indicating minimal stromal damage. Less than fifty percent of them reached a positive medium term success.

Twenty four percent of the **PBSCT** patients were treated with **Bu/Cy**. Only two of the patients produced an early **RMI** response, one died. Three patients produced an early

ANC response; two of them achieved a "12 month post transplant trilineage response". Four produced an early **PLT** response, only one achieved a "12 month post transplant trilineage response". Less than fifty percent of the patients produced early responses in any of the three parameters; this indicated possible stromal damage. The **PLT** was the only parameter with a successful seventy five percent of the patients achieving a "12 month post transplant trilineage response". In summary, most patients treated with **Bu/Cy** appeared to have sustained some stromal damage, less damage was indicated in the megakaryocyte line than either the erythrocytic or granulocytic lines.

Eleven percent of the **PBSCT** patients were preconditioned with **Bu/Mel/Thiotep.** Fifty percent of the patients produced early **RMI** and **ANC** responses, fifty percent died. An early **PLT** response was produced in seventy five of the patients, sixty seven percent of them died. All three parameters generated more than fifty percent early responses, this indicated minimal stromal damage. Less than fifty percent of them achieved a "12 month post transplant trilineage response".

The **Cyclo/TNI** and **Cyclo/ALG** were used on the **SAA** allo**PBSCT** patients, all three generated early **RMI** and **PLT** responses, with one early and one late **ANC** response. **Cyclo/Alg** produced late responses within all three parameters; all three of the patients achieved a "12 month post transplant trilineage response". With these low patient numbers it was difficult to deduce whether **Cyclo/Alg** preconditioning was a direct cause of slower responses. More patients would need to be analysed to draw an adequate conclusion.

Eighty three percent of the **BMT** were treated with **Cyclo/TBI/TNI**. Forty percent of the patients produced an early **RMI** response; fifty percent of them died. Sixty percent of the patients produced early **ANC** response, only thirty three percent achieved a "12 month post transplant trilineage response". Eighty percent of the patients produced **PLT** responses, sixty three percent of them were early, and sixty percent of them achieved a "12 month post transplant trilineage response". Because the **RMI** generated less than a fifty percent early response, and the **ANC** and **PLT** more, perhaps the preconditioning

produced a greater adverse effect on the erythroid line in **BMT**. *The* **PLT** *was the only* parameter to indicate a positive medium term success. Perhaps the megakaryocytic line was less affected by **Cyclo/TBI/TNI** than the other two.

Both the **SAA** allo**BMT** patients that were treated with **Cyclo/TNI** and **Cyclo/Alg,** they generated late responses in all three parameters, both died. With only two cases no accurate assessment could be made.

4.4.5. Mononuclear Count (MNC), Colony Forming Unit – Granulocytic Monocytic (CFU-GM) and Cluster Designation 34+ Count (CD34+) in Peripheral Blood Stem Cell Transplant (PBSCT) and Bone Marrow Transplant (BMT) Patients

The mean **BMT MNC** was 0.97 $x10^8$/kg and **CFU-GM** was 16.75 $x10^4$/kg, the counts were considerably lower than the **PBSCT** at 7.51 $x10^8$/kg for the **MNC** and 28.77 $x10^4$/kg for the **CFU-GM**. There were no **CD34+** counts on the **BMT** patients but the **PBSCT** revealed a mean count of 83.83 (Figure 8 Page 44).

Forty three percent of the **PBSCT** had **MNC** greater than the mean. Seventy five percent of the patients produced an early **RMI** response, only thirty three percent of them achieved a "12 month post transplant trilineage response". Sixty one percent of the patients produced an early **ANC** response, only twenty two percent of them achieved a"12 month post transplant trilineage response". Seventy two percent of the patients produced an early **PLT** response; forty three percent of them achieved a"12 month post transplant trilineage response". These results indicated that greater than average **MNC** produce early responses in all three parameters. Unfortunately no positive prognosis for medium term success was found.

All twelve **BMT** had **MNC** performed. Forty two percent of them produced counts greater than the mean, only twenty five percent of the patients produced an early **RMI** response, two of the three patients achieved a "12 month post transplant trilineage response", the **SAA** patient died. Only seventeen percent generated early **ANC** and **PLT** responses, both achieved a "12 month post transplant trilineage response". Even with very low numbers, higher **MNC** did not appear to generate early responses. Sixty six

percent of the early **RMI** responses, and one hundred percent of the **ANC** and **PLT** responses did however, achieve a "12 month post transplant trilineage response". These findings indicated that a higher **MNC** does not always result in a positive medium term success.

Seventy four percent of the **PBSCT** patients had **CFU-GM** counts performed. Only twenty nine percent of them generated counts greater than the mean. Sixty two percent of the patients produced an early **RMI** response, only forty percent of them achieved a "12 month post trilineage response". Seventy five percent of the patients produced an early **ANC** response, only thirty three percent achieved a "12 month post transplant trilineage response". Eighty eight percent of the patients produced an early **PLT** response, only twenty nine percent achieved a "12 month post transplant trilineage response". Greater than average **CFU-GM** counts appeared to generate early responses in all three parameters, particularly the **PLT.** No positive prognosis was indicated medium term.

Eighty percent of the **BMT** patients had **CFU-GM** counts performed, only thirty percent generated counts greater than the mean. Two patients produced an early **RMI** response, two produced an early **ANC** response and one an early **PLT** response, of the three patients, two died. Unfortunately the numbers were too low for accurate assessment.

There were no **CD34+** counts on any of the **BMT**. All seven were performed on **PBSCT** patients, with only two counts above the mean, both generated early responses in all three parameters, but only one achieved a "12 month post transplant trilineage response", he generated the highest count of all. These patient numbers were too low for accurate assessment.

4.4.6. Response Range for Peripheral Blood Stem Cell Transplant (PBSCT) and Bone Marrow Transplant (BMT) Patients

4.4.6.1. Reticulocyte Maturation Index (RMI)

None of the **BMT** generated **RMI** counts greater than **2SD** within the first forty days, even with a forty percent "12 month post transplant trilineage response". Forty three percent of the patients with **RMI** values below **2SD** were **BMT**, twenty nine percent

(two) of them were **SAA** allo**BMT** with "graft failures", and the remaining fourteen percent achieved a "12 month post transplant trilineage response".

Of the patients with **RMI** values above **2SD** within the first forty days, all of them were **PBSCT**. Twenty percent of the patients fell below **2SD**; eleven percent of them achieved a successful "12 month post transplant trilineage response". As a result an **RMI** value above or below **2SD** did not appear to be significant.

4.4.6.2. Absolute Neutrophil Count (ANC)

Of the eighteen percent of patients with **ANC** above **2SD** within the first forty days, only twelve percent were **BMT**, the other eighty eight percent were **PBSCT**. Thirty nine percent of the patient counts fell below **2SD**, fifteen percent of them were **BMT**, and eighty five percent were **PBSCT**. Although none of the **BMT** achieved a "12 month post transplant trilineage response", eighty eight percent of the **PBSCT** did. Patients with no clinical infection and **ANC** above **2SD** within the first forty days indicated a worthy chance of success.

In the **SAA** transplants, both the **RMI** and **ANC** revealed that when results fall below the expected range on both **BMT** and **PBSCT**, patients appear to be at risk for possible graft failure. Both of the **BMT SAA** "graft failures" produced low **RMI** and **ANC** values.

4.4.6.3. Platelet Count (PLT)

All patients achieved a **PLT** greater than **2SD** within the first forty days, in both the **BMT** and the **PBSCT**. No patients produced **PLT** of zero at any point.

4.4.7. "Day 14" Marrow Biopsy results on Peripheral Blood Stem Cell Transplant (PBSCT) and Bone Marrow Transplant (BMT) Patients

"Day 14" marrow biopsies were performed on eighty three percent of the **BMT** and twenty four percent of the **PBSCT**. These biopsies revealed no additional information in comparison to the "day 28" marrow biopsies.

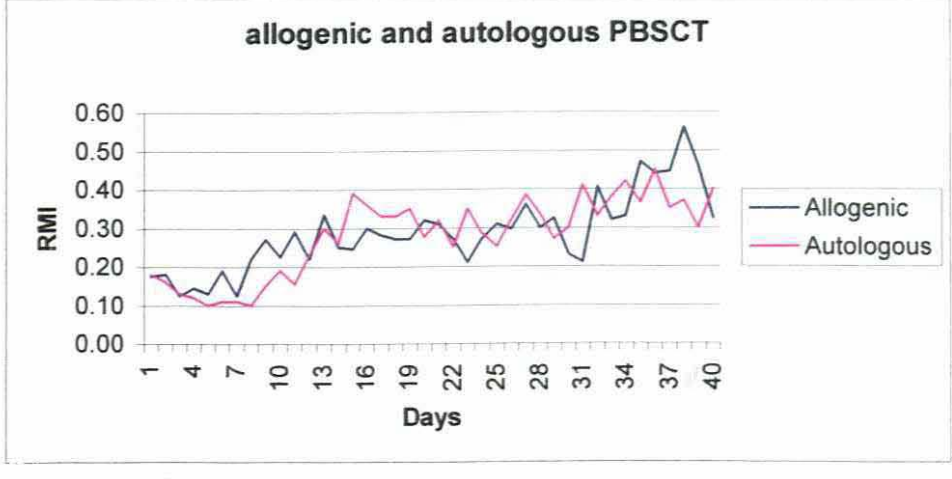
4.5. Comparison of allogenic Peripheral Blood Stem Cell Transplant (alloPBSCT) and autologous Peripheral Blood Stem Cell Transplant (autoPBSCT) Patients

Of the thirty five **PBSCT** analysed, twenty two were allogenic transplants and thirteen were autologous.

4.5.1. The Reticulocyte Maturation Index (RMI) Response

4.5.1.1. The Mean and Median Response Time for the Reticulocyte Maturation Index (RMI) between allogenic Peripheral Blood Stem Cell Transplant (alloPBSCT) and autologous Peripheral Blood Stem Cell Transplant (autoPBSCT) Patients

The allo**PBSCT** mean **RMI** Response Time was eight days, with a range of one to seventeen days (Figure 5 Page 39). The auto**PBSCT** mean Response Time was nine days, with a range of one to seventeen days (Figure 5 Page 39). The allo**PBSCT** median Response Time was eight days with a range of one to fifteen days; the auto**PBSCT** was ten days with a range of one to seventeen days (Figure 6 Page 40). All allo**PBSCT** and auto**PBSCT** responded within seventeen days.



Graph 25: The mean **RMI** Response Time, in days, for twenty two allogenic and thirteen autologous **PBSCT** transplant patients.

An allo**PBSCT** mean **RMI** of 0.33 IU was seen within forty days (Graph 25 Page 92), 0.23 IU at three months, 0.22 IU at four months, 0.19 IU at five months and 0.22 IU at six months, a fifty percent decrease (Figure 10 Page 46). A median of 0.33 IU was seen within forty days, 0.21 IU at three months, 0.23 IU at four months, 0.19 IU at five months and 0.22 IU at six months, a fifty percent decrease (Figure 11 Page 46).

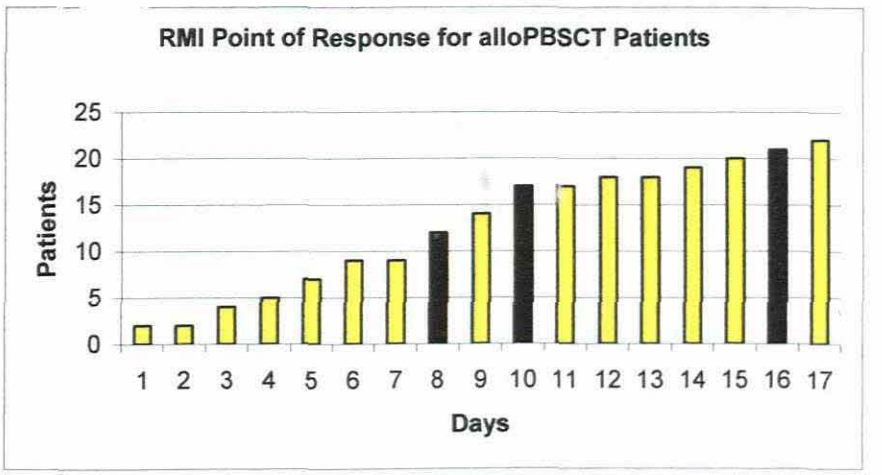
An auto**PBSCT** mean **RMI** of 0.4 IU was seen within forty days (Graph 25 Page 92), 0.30 IU at three months, 0.31 IU at four months, 0.21 IU at five months and 0.16 IU at six months, a one hundred and fifty six percent decrease (Figure 10 Page 46). A median of 0.40 IU was seen at forty days, 0.33 IU at three months, 0.32 IU at four months, 0.21 IU at five months and 0.16 IU at six months, a one hundred and fifty percent decrease (Figure 11 Page 46).

The allo**PBSCT** and auto**PBSCT RMI** values decreased over six months. The allo**PBSCT RMI** produced in a fifty percent decrease. It appeared that the allogenic transplants produced the most stable **RMI** values of all the groups. The auto**PBSCT** group produced the highest mean **RMI** decrease over six months, at one hundred and fifty six percent. Although the auto**PBSCT** appeared to produce the least stable **RMI** values over six months, they generated healthier medium term responders, and seventy seven percent achieved a "12 month post transplant trilineage response", compared to the allo**PBSCT's** twenty percent.

4.5.1.2. The Point of Response for the Reticulocyte Maturation Index (RMI) between the allogenic Peripheral blood Stem Cell Transplant (alloPBSCT) and the autologous Peripheral Blood Stem Cell Transplant (autoPBSCT) Patients.

- **alloPBSCT**

A 95% Point of Response was seen within sixteen days, 75% within ten days and 50% within eight days (Graph 26 Page 94).

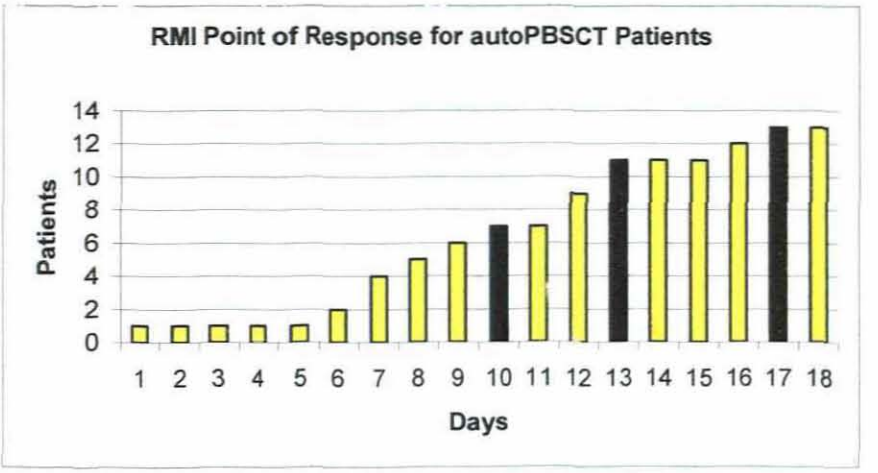


Graph 26: The **RMI** Point of Response, in days, for twenty two allo**PBSCT** patients, 50% 75% and 95% are highlighted.

Ninety five percent of the patients fell within the 95% Point of Response. One patient, an **AML** allo**PBSCT**, responded on day seventeen, he was not considered a "delayed response".

- **autoPBSCT**

A 95% Point of Response was seen within seventeen days, 75% within thirteen days and 50% within ten days (Graph 27 Page 95).



Graph 27: The **RMI** Point of Response, in days, for thirteen auto**PBSCT** patients, 50%, 75% and 95% are highlighted.

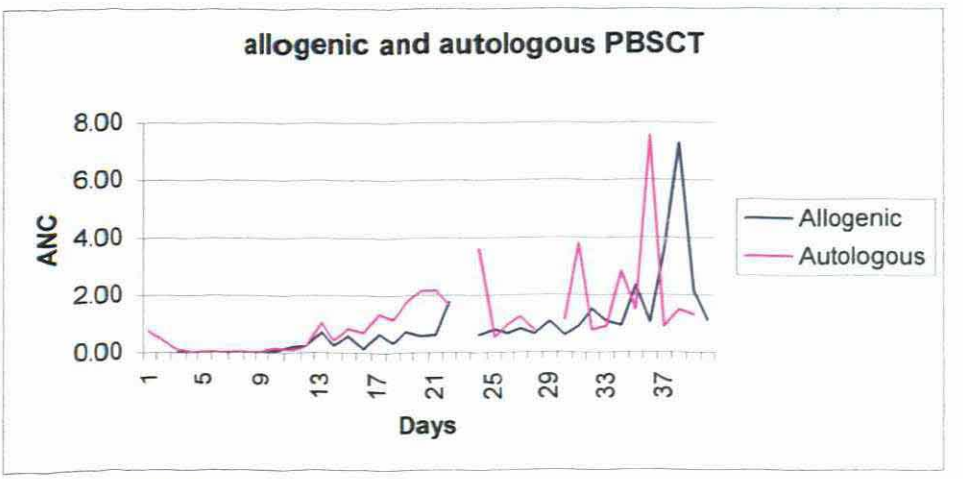
One hundred percent of the patients responded within the 95% Point of Response.

No significant difference was indicated between the allo**PBSCT** and the auto**PBSCT** **RMI** results. Both groups produced a mean Response Time of eight days and a Point of Response of sixteen days.

4.5.2. The Absolute Neutrophil Count (ANC) Response

4.5.2.1. The Mean and Median Response Time for the Absolute Neutrophil Count (ANC) between allogenic Peripheral Blood Stem Cell Transplant (alloPBSCT) and autologous Peripheral Blood Stem Cell Transplant (autoPBSCT) Patients

The mean Response Time for the alloPBSCT was thirteen days, with a range of eight to thirty one days (Figure 5 Page 39). The mean Response Time for the autoPBSCT was fifteen days, with a range of nine to twenty one days (Figure 5 Page 39). The median Response Time for alloPBSCT was thirteen days, with a range of eight to thirty one days (Figure 6 Page 40). The median Response Time for the autoPBSCT was fifteen days, with a range of nine to twenty one days (Figure 6 Page 40). Ninety five percent of the alloPBSCT responded within thirty days and one hundred percent of the autoPBSCT responded within twenty one days.



Graph 28: The mean ANC Response Time, in days, for twenty two allogenic and thirteen autologous PBSCT transplant patients.

An alloPBSCT mean ANC of 1.13 x10^9/l was seen within forty days (Graph 28 Page 96), 2.36 x10^9/l at three months, 2.07 x10^9/l at four months, 2.19 x10^9/l at five months and 2.64 x10^9/l at six months, a one hundred and thirty four percent increase (Figure 12 Page 47). A median of 1.13 x10^9/l was seen within forty days (Graph 28 Page 96), 2.68 x10^9/l

at three months, 1.90 x10^9/l at four months, 1.52 x10^9/l at five months and 2.31 x10^9/l at six months, a one hundred and four percent increase (Figure 11 Page x).

An auto**PBSCT** mean **ANC** of 1.12 x10^9/l was seen within forty days (Graph 28 Page 96), 2.89 x10^9/l at three months, 2.30 x10^9/l at four months, 1.90 x10^9/l at five months and 3.03 x10^9/l at six months, a mean increase of one hundred and seventy one percent (Figure 12 Page 47). A median of 1.30 x10^9/l was seen within forty days, 1.54 x10^9/l at three months, 2.24 x10^9/l at four months, 1.78 x10^9/l at five months and 2.88 x10^9/l at six months, an increase of one hundred and twenty two percent (Figure 13 Page 47).

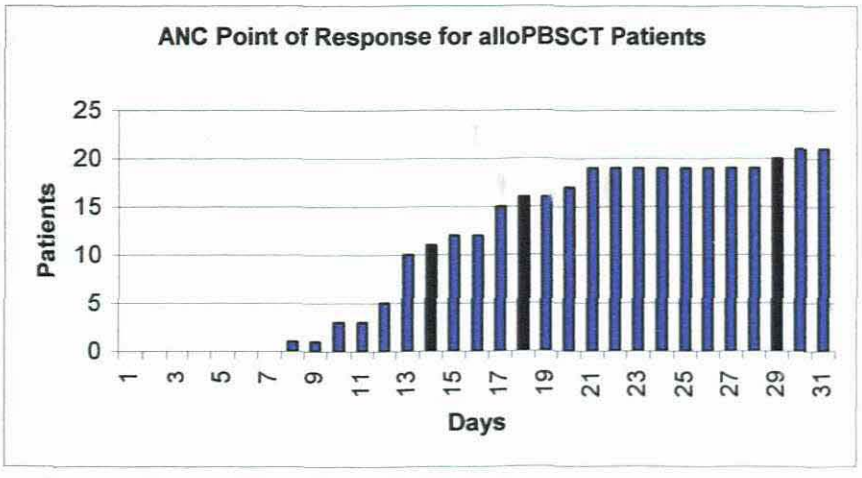
All the allo**PBSCT** patients responded within thirty days and the auto**PBSCT** within twenty one days. The **RMI** mean Response Time was five days and seven days earlier respectively in the allo**PBSCT** and the auto**PBSCT** compared to the **ANC** response. Although the allo**PBSCT** recovered two days earlier compared to the auto**PBSCT**, the auto**PBSCT** produced a healthier response within six months with a twenty eight percent higher count.

The **ANC PBSCT** results for the first forty days were not graphically as steady as the **RMI** (Graph 28 Page 96). Although the allo**PBSCT** group produced a mean Response Time three days prior to the auto**PBSCT** group, within forty days both produced similar counts. The auto**PBSCT** was the healthier responder within six months with a twenty eight percent higher count than the allo**PBSCT**. The auto**PBSCT** group responded second to the **CML** allo**PBSCT** group in increasing values. The auto**PBSCT** produced the higher **ANC** increase over the six months, as well as the most significant **RMI** decrease.

4.5.2.2. The Point of Response for the Absolute Neutrophil Count (ANC) between allogenic Peripheral Blood Stem Cell Transplant (alloPBSCT) and autologous Peripheral Blood Stem Cell Transplant (autoPBSCT) Patients.

- **alloPBSCT**

A 95% Point of Response was seen within twenty nine days, 75% within eighteen days and 50% within fourteen days (Graph 29 Page 97).



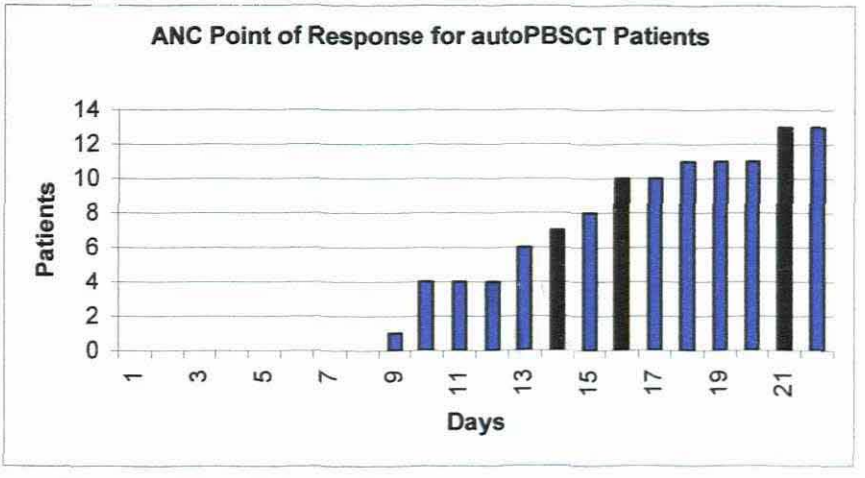
Graph 29: The **ANC** Point of Response, in days, for twenty allo**PBSCT** patients, 50%, 75% and 95% are highlighted.

Ninety four percent of the allo**PBSCT** patients fell within 95% Point of Response. One patient an **AML** allo**PBSCT** responded on day thirty, and was not considered a "delayed response". One patient, a **CML** allo**PBSCT** who responded on day fifty seven, was considered a "delayed response".

The **RMI** allo**PBSCT** Point of Response was fourteen days earlier than the **ANC**, indicating an early erythroid recovery.

- **autoPBSCT**

A 95% Point of Response was seen within twenty one days, 75% within sixteen days and 50% within fourteen days (Graph 30 Page 99).



Graph 30: The **ANC** Point of Response, in days, for thirteen auto**PBSCT** patients, 50%, 75% and 95% are highlighted.

All the auto**PBSCT** patients fell within 95% Point of Response.

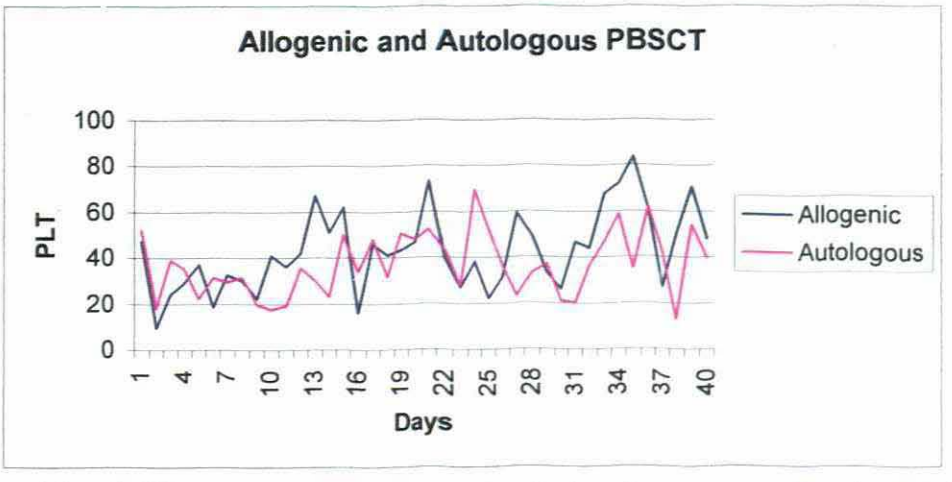
The auto**PBSCT ANC** Point of Response was eight days earlier than the allo**PBSCT**. The autologous transplants as a collective, indicated a "pattern of recognition". The autologous transplant group produced a nine day earlier Point of Response compared to the allogenic transplant group, this continued throughout the six month period resulting in a higher percentage increase compared to the allo**PBSCT**. The last of the patients to respond were the **CML** allo**PBSCT**, this indicated that **CML** patients are the slowest responders post transplant.

The auto**PBSCT RMI** Point of Response was five days earlier than the **ANC** response, indicating earlier erythroid recovery.

4.5.3. The Platelet Count (PLT) Response

4.5.3.1. The Mean and Median Response Time for the Platelet Count (PLT) between allogenic Peripheral Blood Stem Cell Transplant (alloPBSCT) and autologous Peripheral Blood Stem Cell Transplant (autoPBSCT) Patients

The mean Response Time for the allo**PBSCT** was twelve days, with a range of seven to thirty six days. The auto**PBSCT** produced a mean of seventeen days, with a range of ten to twenty eight days (Figure 5 Page 39). The median Response Time for the allo**PBSCT** was twelve days, with a range of seven to thirty three days. The auto**PBSCT** median was fifteen days, with a range of ten to twenty eight days (Figure 6 Page 40). All the allo**PBSCT** patients responded within thirty six days and all the auto**PBSCT** responded within twenty eight days.



Graph 31:The median **PLT** Response Time, in days, for twenty two allogenic and thirteen autologous **PBSCT** transplant patients.

An allo**PBSCT** mean **PLT** of 48 $x10^9$/l was seen within forty days (Graph 31 Page 100), 115 $x10^9$/l at three months, 147 $x10^9$/l at four months, 145 $x10^9$/l at five months and 157 $x10^9$/l at six months, a mean increase of two hundred and twenty seven percent (Figure 14 Page 48). A median of 48 $x10^9$/l was seen within forty days (Graph 31 Page 100), 107 $x10^9$/l at three months, 160 $x10^9$/l at four months, 126 $x10^9$/l at five months and 165

$x10^9/l$ at six months, a median increase of two hundred and forty four percent (Figure 15 Page 48).

An auto**PBSCT** mean **PLT** of 40 $x10^9/l$ was seen within forty days (Graph 31 Page 100), 64 $x10^9/l$ at three months, 77 $x10^9/l$ at four months, 92 $x10^9/l$ at five months and 115 $x10^9/l$ at six months, a mean increase of one hundred and eighty eight percent (Figure 14 Page 47). A median of 48 $x10^9/l$ was seen within forty days (Graph 31 Page 100), 70 $x10^9/l$ at three months, 72 $x10^9/l$ at four months, 73 $x10^9/l$ at five months and 107 $x10^9/l$ at six months, an increase of one hundred and sixty eight percent (Figure 15 Page 48).

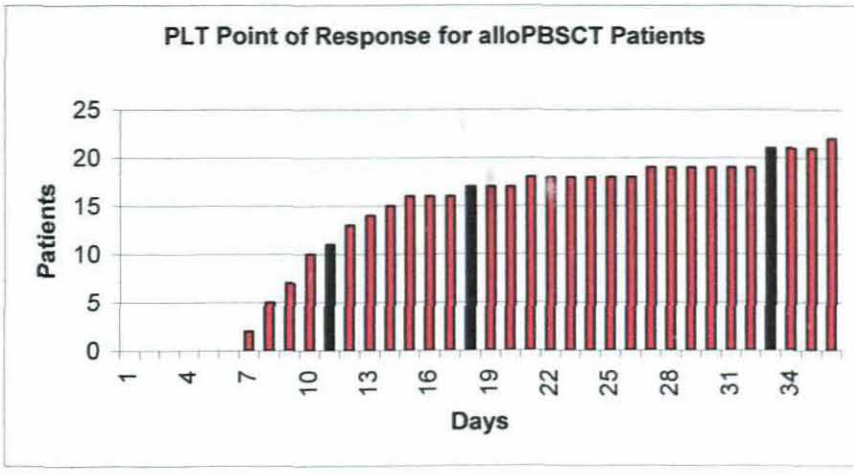
The **RMI** mean Response Time was five days and nine days earlier than the **PLT** respectively for allo**PBSCT** and auto**PBSCT**. The **ANC** mean Response Time was one day earlier in the allo**PBSCT** and two days earlier in the auto**PBSCT**.

The **PLT** was similar for both allo**PBSCT** and auto**PBSCT** at forty days. The allo**PBSCT**, however, produced a healthier percentage response within six months at two hundred and twenty seven percent, the highest increase of all the groups. This indicated that the **PLT** response was, unlike the **ANC** response, possibly due to the effect of the preconditioning, **MNC** or **CFU-GM** counts.

4.5.3.2. The Point of Response for the Platelet Count (PLT) between allogenic Peripheral Blood Stem Cell Transplant (alloPBSCT) and autologous Peripheral Blood Stem Cell Transplant (autoPBSCT) Patients.

- **alloPBSCT**

A 95% Point of Response was seen within thirty three days, 75% eighteen days and 50% eleven days (Graph 32 Page 102).



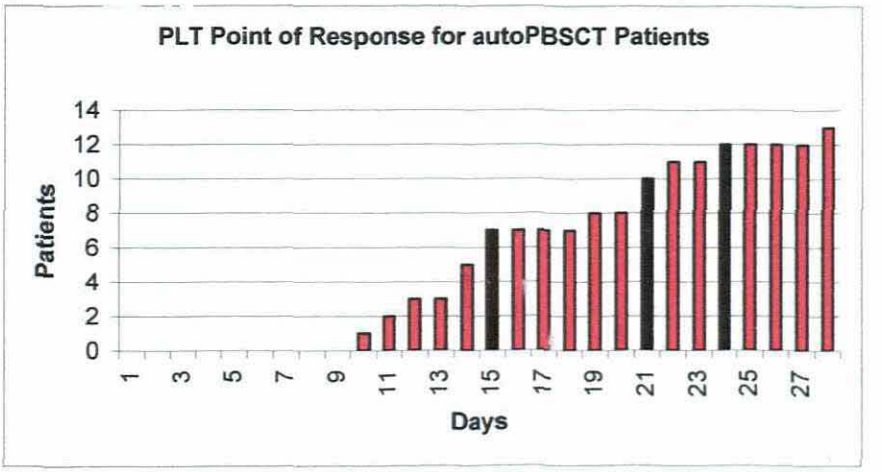
Graph 32: The **PLT** Point of Response, in days, for twenty one allo**PBSCT** patients, 50%, 75% and 95% are highlighted.

Ninety five percent of the patients fell within 95% Point of Response. A **CML** allo**PBSCT,** who responded on day thirty six, was considered a "slightly delayed response".

The **RMI** Point of Response was seventeen days prior to the **PLT** response. The **ANC** response was seen three days earlier than the **PLT** response. This indicated an early erythroid recovery.

- **autoPBSCT**

A 95% Point of Response was seen within twenty four days 75% within twenty one days and 50% within fifteen days (Graph 33 Page 103).



Graph 33: The **PLT** Point of Response, in days, for twelve auto**PBSCT** patients, 50%, 75% and 95% are highlighted.

Ninety five percent of the patients fell within 95% Point of Response. One patient an **AML** auto**PBSCT**, who responded on day twenty eight, was considered a "slightly delayed response". He also fell outside the limits for the autologous transplants but fell within limits for the total transplant group.

The **RMI** Point of Response was seen ten days earlier than the **PLT** response. The **ANC** was seen three days earlier than the **PLT** response. Like the allo**PBSCT** a significantly earlier **RMI** response was seen in comparison to the **ANC** and **PLT** counts. This indicated an earlier erythroid recovery.

The **PLT** auto**PBSCT** was seen nine days earlier than the allo**PBSCT**, unlike the Response Time, where the allo**PBSCT** mean was earlier than the auto**PBSCT** by five

*days. Like the allo***PBSCT** *the auto***PBSCT** *produced a later* **ANC** *and* **PLT**, *but were* similar compared to the **RMI** Point of Response.

The **PLT** allo**PBSCT** Point of Response increased steadily within the first thirty three days. The auto**PBSCT** Point of Response like the other autologous groups were of the earliest to respond. This was possibly due to the autologous transplant "pattern of recognition".

The **PLT** auto**PBSCT** mean Response Time, unlike the **ANC** mean response was seen *earlier than the allo***PBSCT.** *Both* **ANC** *and* **PLT** *produced an earlier Point of* Response in the auto**PBSCT**, possibly due to a "pattern of recognition". The mean percentage increase of **ANC** for the auto**PBSCT** over six months was reversed in the **PLT**, with the allo**PBSCT** being superior. This was possibly due either to the preconditioning, **MNC** or **CFU-GM** counts.

4.5.4. Preconditioning in allogenic Peripheral Blood Stem Cell Transplant (alloPBSCT) and autologous Peripheral Blood Stem Cell Transplant (autoPBSCT) Patients

Twenty three allo**PBSCT** patients were preconditioned; treatment was as follows:

- Sixteen **Cyclo/TBI/TNI.**
- Three **By/Cy.**
- One **Bu/Mel/Thiotep.**
- Two **Cyclo/TNI.**
- One **Cyclo/Alg.**

Fourteen auto**PBSCT** patients were preconditioned; treatment was a follows:

- Three **Bu/Mel/Thiotep.**
- Six **Bu/Cy.**
- Five **Cyclo/TBI/TNI.**

Seventy percent of the allo**PBSCT** were preconditioned with **Cyclo/TBI/TNI.** Sixty nine percent of them produced an early **RMI** response; twenty seven percent of those achieved a "12 month post transplant trilineage response". Sixty three percent of the patients produced early **ANC** and **PLT** responses; twenty percent in each parameter achieved a "12month post transplant trilineage response". Although more than fifty percent of the patients generated early **RMI**, **ANC** and **PLT** responses, indicating minimal stromal damage, very few achieved a positive medium term success.

Only thirteen percent (three) of the allo**PBSCT** were preconditioned with **Bu/Cy**. All the patients produced greater than average early responses in all three parameters, only one achieved a "12 month post transplant trilineage response". Two of the **SAA** patients were treated with **Cyclo/TNI**, one generated early responses in all three parameters the other failed to; both achieved a "12 month post transplant trilineage response". The patient that was treated with **Bu/Mel/Thiotep** generated late responses in all three parameters died. None of the three preconditioning combinations contained sufficient patient numbers for an accurate assessment.

Only thirty six percent of the auto**PBSCT** were preconditioned with **Cyclo/TBI/TNI**, eighty percent achieved a "12 month post transplant trilineage response". Fifty percent of them produced an early response in all three parameters and achieved a "12 month post transplant trilineage response". The auto**PBSCT** appeared to have sustained only minimal stromal damage, particularly due to the positive medium term prognosis.

Forty three percent of the auto**PBSCT** patients were treated with **Bu/Cy,** fifty percent achieved a "12 month post transplant trilineage response", and only one patient produced an early **RMI**, two, an early **ANC** and one an early **PLT** response. Even with low patient numbers, an early recovery was not seen with this preconditioning regime, although there was a positive medium term prognosis.

Twenty one percent of the auto**PBSCT** patients were treated with **Bu/Mel/Thiotep**, the patient with early responses in all three parameters died. Of the two patients that

achieved a "12 month post transplant trilineage response", one generated early responses in all three parameters the other only in the **PLT**. Due to low patient numbers no accurate assessment was made.

4.5.5. Mononuclear Count (MNC), Colony Forming Unit – Granulocytic Monocytic (CFU-GM) and Cluster Designation 34+ Counts (CD34+) in allogenic Peripheral Blood Stem Cell Transplant (alloPBSCT) and autologous Peripheral Blood Stem Cell Transplant (autoPBSCT) Patients

MNC were performed on all the allo**PBSCT**, with a mean of 7.93 $x10^8$/kg. All of the auto**PBSCT** had **MNC**, with a mean of 6.53 $x10^8$/kg. Eighty three percent of the allo**PBSCT** had **CFU-GM** counts, with a mean of 31.25 $x10^4$/kg. Only sixty two percent of the auto**PBSCT** had **CFU-GM** counts, with a mean of 22.15 $x10^4$/kg. Only twenty five percent of the allo**PBSCT** had **CD34+** counts, with a mean of 62.35 and fifteen percent of the auto**PBSCT** patients **CD34+** counts, with a mean of 137.55 (Figure 8 Page 44).

Forty six percent of the allo**PBSCT** produced **MNC** greater than the mean. Fifty five percent of these patients generated an early **RMI** response, and only forty percent of them achieved a "12 month post transplant trilineage response". Sixty four percent of the patients generated an early **ANC** response; twenty eight percent of them achieved a "12 month post transplant trilineage response". Sixty four percent of the patients generated an early **PLT** response; forty three percent of them achieved a "12 month post transplant trilineage response". More than fifty percent of the patients with **MNC** greater than the mean generated early **RMI**, **ANC** and **PLT** responses. As a result higher **MNC** yielded early responses. Unfortunately, less than fifty percent of the patients achieved a "12 month post transplant trilineage response"; this indicated a less positive medium term prognosis.

Only twenty nine percent of the patients produce **MNC** greater than the mean in the auto**PBSCT.** Fifty percent of the patients generated an early **RMI** and **PLT** response; fifty percent of them achieved a "12 month post transplant trilineage response". There were no early **ANC** responses. This indicated that higher **MNC** resulted in early **RMI**

and **PLT** responses. Thirty six percent of all the auto**PBSCT** with below average **MNC** achieved a "12 month post transplant trilineage response". Ultimately auto**PBSCT** encompassed more patients with a positive medium term prognosis, regardless of the **MNC**.

CFU-GM counts were performed on eighty three percent of the allo**PBSCT**, forty five percent of them produced counts greater than the mean. Forty four percent of the patients produced early **RMI** and **ANC** responses, all of them died. Seventy seven percent of the patients produced an early **PLT** response; twenty nine percent (two patients) achieved a "12 month post transplant trilineage response". Even with low patient numbers, higher **CFU-GM** counts appear to have little effect on early **RMI** and **ANC** responses, but there was an effect on the **PLT** parameter. Unfortunately, higher **CFU-GM** counts appear to have little to no effect on medium term success.

Only fifty seven percent of the auto**PBSCT** patients had **CFU-GM** counts. One patient produced a count above the mean, which was possibly skewed, as the count was 102.90 $x10^4$/kg. She achieved a "12 month post transplant trilineage response", as well as another sixty four percent of the group. The patient numbers were too low for accurate assessment.

Not enough patients had **CD34+** counts for analysis. The allo**PBSCT** group contained seventy one percent of the counts, with a mean of 62.35. The two patients with counts higher than the mean responded early in all three parameters, one died and the other achieved a "12 month post transplant trilineage response". Two of the auto**PBSCT** patients produced counts resulting in a wide discrepancy; both achieved a "12 month post transplant trilineage response".

4.5.6. Response Range for allogenic Peripheral Blood Stem Cell Transplant (alloPBSCT) and autologous Peripheral Blood Stem Cell Transplant (autoPBSCT) Patients

4.5.6.1. Reticulocyte Maturation Index (RMI)

Of the patients with **RMI** values greater than **2SD** within the first forty days, eighty three percent were allo**PBSCT** and seventeen percent were auto**PBSCT**. One patient achieved a "12 month post transplant trilineage response", he was an auto**PBSCT**. Forty three percent of the allo**PBSCT** fell below **2SD** within the first forty days, with one "12 month post transplant trilineage response".

4.5.6.2. Absolute Neutrophil Count (ANC)

Of the patients with **ANC** greater than **2SD** within the first forty days, eighty eight percent were auto**PBSCT** and thirteen percent were allo**PBSCT**. Eighty six percent of the patients achieved a successful "12 month post transplant trilineage response". It appeared those patients, particularly, within the auto**PBSCT** with an **ANC** greater than **2SD** within the first forty days, devoid of infection, achieved an improved chance of success.

4.5.6.3. Platelet Count (PLT)

Most patients achieved a **PLT** value greater than **2SD** at one point within the first forty days. No patient's **PLT** counts fell to zero.

4.5.7. "Day 14" marrow biopsy Results on allogenic Peripheral Blood Stem Cell Transplant (alloPBSCT) and autologous Peripheral Blood Stem Cell Transplant (autoPBSCT) Patients

Only twenty seven percent of the allo**PBSCT** and eight percent of the auto**PBSCT** had "day 14" marrow biopsies. These biopsies revealed no additional information than the "day 28" marrow biopsy. "Day 14" marrow biopsies proved to be unnecessary provided the peripheral blood counts are taken into account for the first twenty eight days.

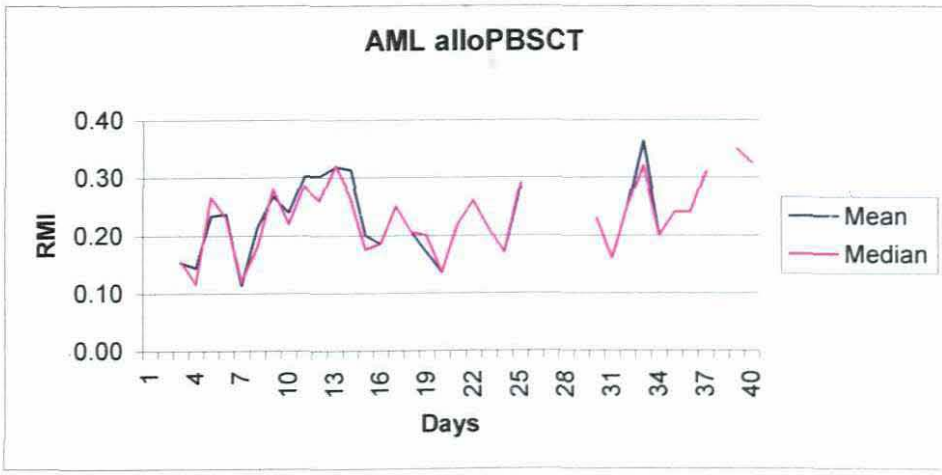
4.6. Acute Myeloid Leukaemia allogenic Peripheral Blood Stem Cell Transplant (AML alloPBSCT) Patients

There were eight allo**PBSCT AML** patients.

4.6.1. The Reticulocyte Maturation Index (RMI) Response

4.6.1.1. The Mean and Median Response Time for the Reticulocyte Maturation Index (RMI) in Acute Myeloid Leukaemia allogenic Peripheral Blood Stem Cell Transplant (AML alloPBSCT) Patients

The mean (Figure 5 Page 39) and a median (Figure 6 Page 40) Response Time was seven days, with a range of one to seventeen days. All the patients responded within seventeen days.



Graph 34: The mean and median **RMI** Response Time, in days, for eight **AML** allo**PBSCT** patients.

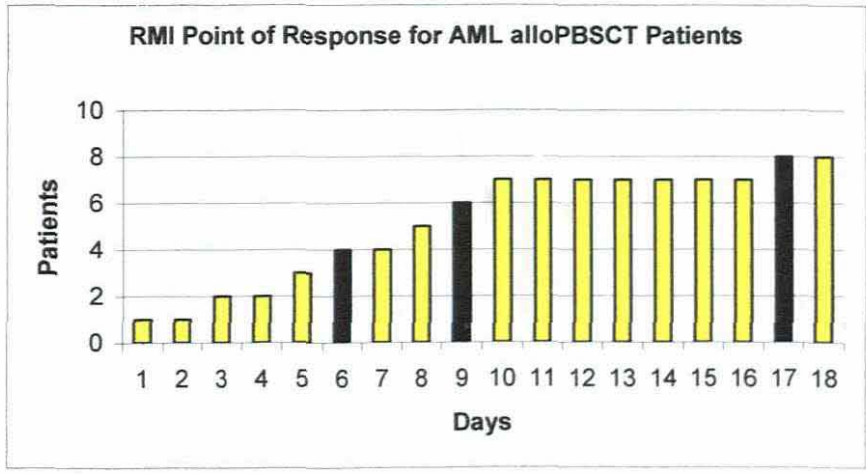
A mean **RMI** of 0.33 IU was seen within forty days (Graph 34 Page 109), 0.25 IU at three months, 0.19 IU at four months, 0.18 IU at five months and 0.16 IU at six months, a mean decrease of ninety four percent (Figure 10 Page 46). The median **RMI** at day forty was 0.33 IU (Graph 34 Page 109), 0.22 IU at three months, 0.21 IU at four months, 0.19 IU at five months and 0.17 IU at six months, a median decrease of ninety four percent (Figure 11Page 46).

Even with small patient numbers within the group, the mean and median **RMI** Response Times for the **AML** allo**PBSCT** were the fastest of all the groups. The **RMI** like all the other groups revealed a steady mean decrease during the six months of ninety four

percent. Even with one of the lowest **RMI** values at day forty and at six months, twenty nine percent of the patients achieved a "12 month post transplant tri lineage response".

4.6.1.2. The Point of Response for the Reticulocyte Maturation Index (RMI) in Acute Myeloid Leukaemia allogenic Peripheral Blood Stem Cell Transplant (AML alloPBSCT) Patients

A 95% Point of Response was seen within seventeen days, 75% within nine days and 50% within six days (Graph 35 Page 110).



Graph 35: The **RMI** Point of Response, in days, for eight **AML** allo**PBSCT** patients, 52%, 75% and 95% are highlighted.

Ninety five percent of the patients responded within 95% Point of Response. One patient responded on day seventeen, he was not considered a "delayed response".

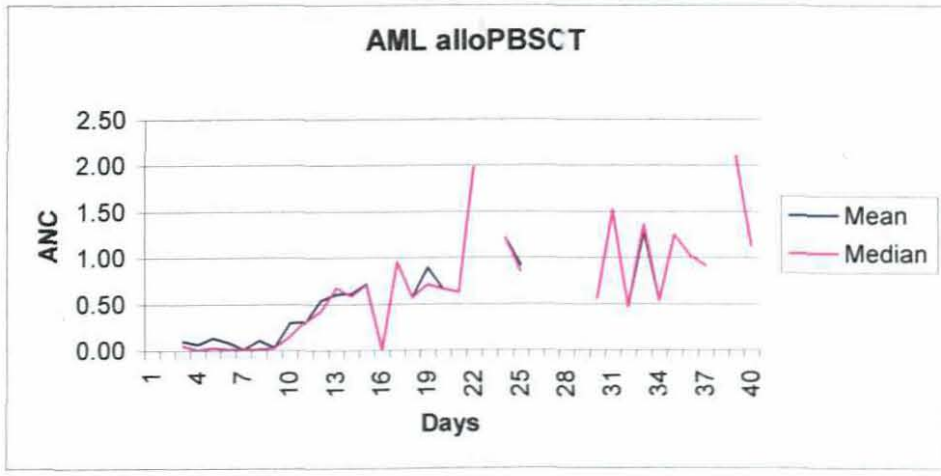
The **RMI** mean and median Response Time did not reflect the same results as the Point of Response. Although the mean **RMI** Response Time was only seven days, the **RMI** Point of Response was seventeen days. The last patient who responded on day seventeen responded seven days later than the patient prior to him. This feature possibly skewed the expected Point of Response for the group, because eighty eight percent of the patients responded within ten days.

The **AML** allo**PBSCT** group produced the earliest Response Time. Unfortunately a very poor success rate of only twenty five percent of them achieved a "12 month post transplant trilineage response".

4.6.2. The Absolute Neutrophil Count (ANC) Response

4.6.2.1. The Mean and Median Response Time for the Absolute Neutrophil Count (ANC) in Acute Myeloid Leukaemia allogenic Peripheral Blood Stem Cell Transplant (AML alloPBSCT) Patients

A mean Response Time of fourteen days (Figure 5 Page 39) and a median of thirteen days (Figure 6 Page 40) were seen, ranging from eight to thirty days. All the patients responded within thirty days.



Graph 36: The mean and median **ANC** Response Time, in days, for eight **AML** allo**PBSCT** patients.

A mean **ANC** of 1.13 $x10^9$/l was seen within forty days (Graph 36 Page 111), 3.10 at three months, 2.41 $x10^9$/l at four months, 1.62 $x10^9$/l at five months and 2.78 10^9/l at six months, a one hundred and forty six percent increase (Figure 12 Page 47). A median of 1.13 $x10^9$/l was seen within forty days (Graph 36 Page 111), 2.87 $x10^9$/l at three months, 1.67 $x10^9$/l at four months, 1.45 $x10^9$/l at five months and 2.05 $x10^9$/l at six months, an eighty one percent increase (Figure 13 Page 47).

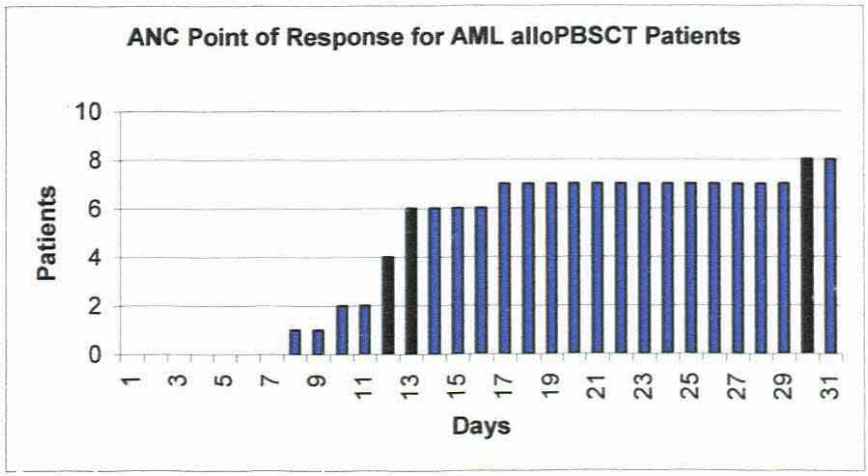
An **ANC** mean Response Time of fourteen days was the earliest of all the groups. An initial **ANC** from forty days to three months of one hundred and seventy four percent was

seen, followed by a decrease over the next two months. A recovery within six months of one hundred and forty six percent from day forty was seen. Sixty four percent of the groups analysed resulted in an **ANC** decrease between four and five months; the **AML** allo**PBSCT** group was the highest at forty nine percent. This feature might be significant due to the poor medium term recovery documented within the allogenic transplants.

The **ANC** mean increase at six months was double that of the median. This feature was possibly due to one patient with a poor response during the six month period, causing overall fluctuations.

4.6.2.2. The Point of Response for the Absolute Neutrophil Count (ANC) in Acute Myeloid Leukaemia allogenic Peripheral Blood Stem Cell Transplant (AML alloPBSCT) Patients

A 95% Point of Response was seen within thirty days, 75% response within thirteen days and a 50% response within twelve days (Graph 37 Page 112).



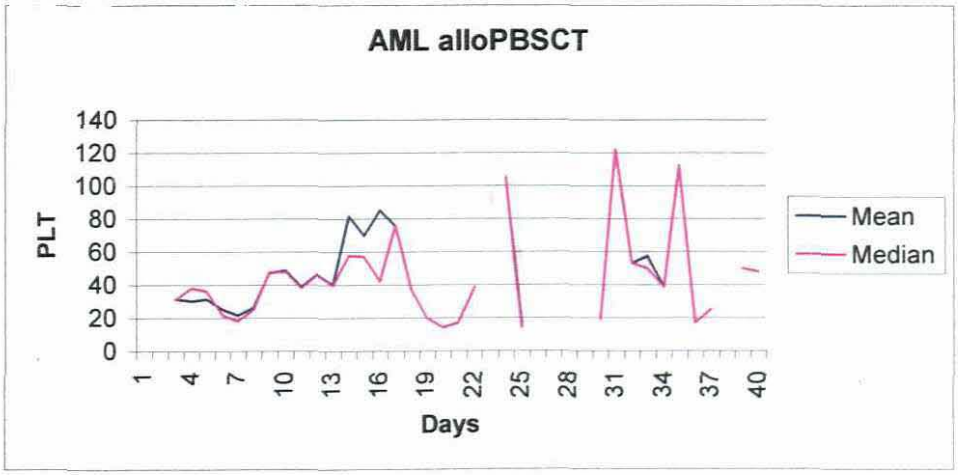
Graph 37: The **ANC** Point of Response, in days, for eight **AML** allo**PBSCT** patients, 50%, 75% and 95% are highlighted.

One hundred percent of the patients responded within 95% Point of Response. The **RMI** Point of Response was thirteen days earlier than the **ANC, which** indicated an earlier erythroid recovery. The last patient to respond on day thirty, responded fourteen days after the patient prior to him. He was not the same patient that responded last in the **RMI** parameter. Nothing of significance was gained from these results.

4.6.3. The Platelet Count (PLT) Response

4.6.3.1. The Mean and Median Response Time for the Platelet Count (PLT) in Acute Myeloid Leukaemia peripheral Blood Stem Cell Transplant (AML alloPBSCT) Patients

A mean Response time of fourteen days (Figure 5 Page 39) and median of eleven days (Figure 6 Page 40) was seen with a range of eight to thirty three days. All the patients responded within thirty three days.



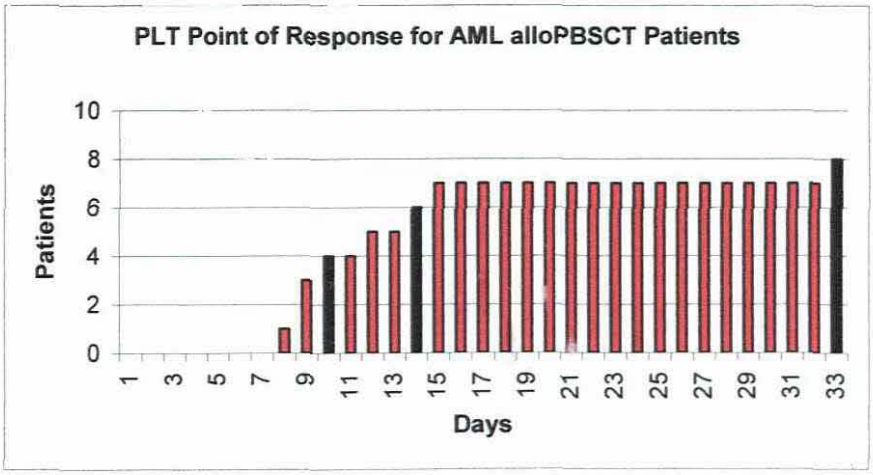
Graph 38: The mean and median **PLT** Response Time, in days, for eight **AML** allo**PBSCT** patients.

A mean **PLT** of 48 x10^9/l was seen within forty days (Graph 38 Page 113), 123 x10^9/l at three months, 152 x10^9/l at four months, 111 x10^9/l at five months and 133 x10^9/l at six months, a mean increase of one hundred and seventy seven percent (Figure 14 Page 48). A median of 48 x10^9/l was seen within forty days (Graph 38 Page 113), 107 x10^9/l at three months, 160 x10^9/l at four months, 126 x10^9/l at five months and 165 x10^9/l at six months, a median increase of two hundred and forty percent (Figure 15 Page 48).

The mean Response Time for the **RMI** was thirteen days prior to the **PLT** response, and the **ANC** was eleven days earlier indicating earlier erythroid recovery. The **PLT** decreased between four and five months by thirty seven percent. The **PLT** indicated the same apparent difference between the mean and median counts within the following six months. This pattern was possibly due to count fluctuations throughout six months.

4.6.3.2. The Point of Response for the Platelet Count (PLT) in Acute Myeloid Leukaemia allogenic Peripheral Blood Stem Cell Transplant (AML alloPBSCT) Patients

A 95% Point of Response was seen within thirty two days, 75% within fourteen days and 50% within ten days (Graph 39 Page 114).



Graph 39: The **PLT** Point of Response, in days, for eight **AML** allo**PBSCT** patients, 50%, 75% and 95% are highlighted.

One hundred percent of the patients responded within 95% Point of Response. The last patient to respond on day thirty two responded eighteen days after the patient prior to him. The breach between the two was possibly due to low patient numbers within the group.

The **RMI** Point of Response was fifteen days earlier than the **PLT** response compared to the **ANC** response, which was only two days earlier indicating early erythroid recovery. The **PLT** Point of Response revealed nothing of significance.

4.6.4. Preconditioning in Acute Myeloid Leukaemia allogenic Peripheral Blood Stem Cell Transplant (AML alloPBSCT) Patients

All eight patients were preconditioned:

- Seven **Cyclo/TBI/TNI.**
- One **Bu/Cy.**

Eighty eight percent of the patients were treated with **Cyclo/TBI/TNI**. Fifty seven percent of the patients produced an early **RMI** response, three patients died and one achieved a "12 month post transplant trilineage response". Eighty six percent of the patients produced an early **ANC** response; sixty six percent of them died. Seventy one percent of the patients produced an early **PLT** response; sixty percent of them died. Although more than fifty percent of the patients generated early responses the **ANC** and **PLT** were the two parameters with the highest percentage early responses. This indicated minimal stromal damage, although the medium term projection for success was extremely poor. There was only one patient treated with **Bu/CY.** He produced an earlier **PLT** response but died. No accurate assessment could be made.

4.6.5. Mononuclear Count (MNC), Colony Forming Unit _ Granulocytic Monocytic (CFU-GM) and Cluster Designation 34+ Counts (CD34+) in Acute Myeloid Leukaemia allogenic Peripheral *Blood Stem Cell Transplant (AML alloPBSCT) Patients*

MNC were performed on all eight patients, with a mean of 7.96 $x10^8$/kg. **CFU-GM** counts were performed on eighty eight percent of the patients, with a mean of 18.15 $x10^4$/kg and only thirteen percent of the patients had **CD34+** counts, with a mean of 34.22. Even with one of the lowest mean **CFU** counts, the **AML** allo**PBSCT** group was still the group with the fastest median **RMI** Response Time.

Sixty three percent of the patients with greater than average **MNC** generated early responses, forty percent (two patients) produced an early **RMI** response, however both died. One hundred percent of the patients produced early **ANC** and **PLT** responses, although eighty percent died. The **ANC** and **PLT** generated the highest percentage of early responses, of all the groups analysed. These findings indicated that the erythroid line was not affected by higher **MNC** where the granulocytic and megakaryocytic lines were. Unfortunately the medium term projection of success for the group was poor.

CFU-GM counts were performed on eighty eight percent of the patients. Only one patient generated counts above the group mean, however he died. The patient numbers were too low for an accurate assessment.

4.6.6. Response range for Acute Myeloid Leukaemia allogenic Peripheral Blood Stem Cell Transplant (AML alloPBSCT) Patients

4.6.6.1. Reticulocyte Maturation Index (RMI)

One patient obtained **RMI** values greater than **2SD** within the first forty days, and one patient below, however both patients died. Patient numbers were too low for an accurate assessment.

4.6.6.2. Absolute Neutrophil Count (ANC)

No patients obtained **ANC** greater than **2SD** within the first forty days. This was an interesting fact considering that thirty percent of the **AML** allo**PBSCT** achieved a "12 *month post transplant trilineage response*". *Thirty percent of the patients produced* **ANC** below **2SD** within the first forty days; fifty percent achieved a "12 month post transplant trilineage response". Even with low patient numbers the **AML** allo**PBSCT** group indicated that higher **ANC** is not necessarily an indicator for medium term success.

4.6.6.3. Platelet Count (PLT)

All the patients obtained **PLT** counts greater than **2SD** within twelve days. The patient to achieve a "12 month post transplant trilineage response" was the only one with a **PLT** greater than 50 $x10^9/l$ on the first day post transplant. None of the patients had **PLT** fall to zero within the first forty days.

4.6.7. "Day 14" marrow biopsy results on Acute Myeloid Leukaemia allogenic Peripheral Blood Stem Cell Transplant (AML alloPBSCT) Patients

"Day 14" marrow biopsies were performed on thirty eight percent of the patients. They all indicated engraftment at both "day 14" and "day 28". One patient who revealed no erythroid or megakaryocytic engraftment at "day 28", might have benefited from a day "14 marrow". However, early **RMI, ANC** and **PLT** responses were seen. Although the patient died, these findings indicated the importance of including peripheral blood counts post transplant.

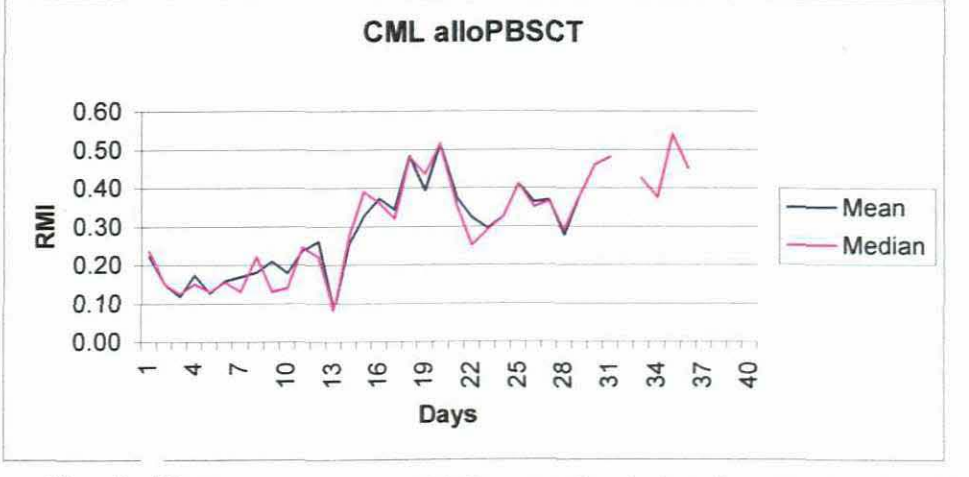
4.7. Chronic Myeloid Leukaemia allogenic Peripheral Blood Stem Cell Transplant (CML alloPBSCT) Patients

There were seven **CML** allo**PBSCT** analysed, showing a one hundred percent **RMI** and **PLT** response within sixteen and forty days respectively, only an eighty six percent **ANC** response was seen within forty days.

4.7.1. The Reticulocyte Maturation Index (RMI) Response

4.7.1.1. The Mean and Median Response Time for the Reticulocyte Maturation Index (RMI) in Chronic Myeloid Leukaemia allogenic Peripheral Blood Stem Cell Transplant (CML alloPBSCT) Patients

The mean Response Time was eleven days, with a range of five to sixteen days (Figure 5 Page 39). The median Response Time was twelve days, with a range of five to sixteen days (Figure 6 Page 40). All the patients responded within sixteen days.



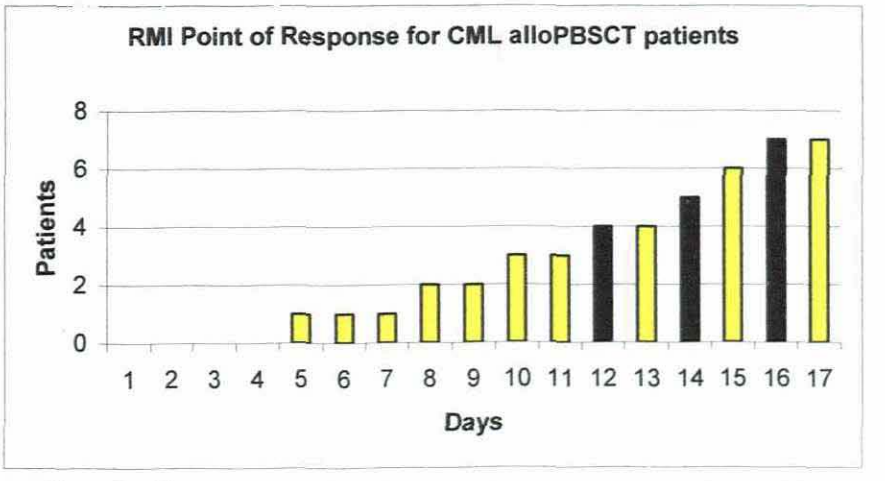
Graph 40: The mean and median **RMI** Response Time, in days, for seven **CML** allo**PBSCT** patients.

A **CML** allo**PBSCT** mean **RMI** of 0.45 IU was seen within thirty six days (Graph 40 Page 117), 0.22 IU at three months, 0.26 IU at four months, 0.21 IU at five months and 0.28 IU at six months, a mean decrease of sixty percent (Figure 10 Page 46). A median of 0.45 IU was seen within thirty six days (Graph 40 Page 117), 0.20 IU at three months, 0.26 IU at four months, 0.20 IU at five months and 0.33 IU at six months, a mean decrease of thirty six percent (Figure 11 Page 46).

The **CML** allo**PBSCT RMI** Response Time was the longest of all the groups analysed. This indicated later recoveries within **CML** transplants. A sixty percent decrease in **RMI** values was seen within six months. This revealed nothing additional compared to any of the other groups.

4.7.1.2. The Point of Response for the Reticulocyte Maturation Index (RMI) in Chronic Myeloid Leukaemia allogenic Peripheral Blood Stem Cell Transplant (CML alloPBSCT) Patients

A 95% Point of Response was seen within sixteen days, 75% response within fourteen days and 50% response with twelve days (Graph 41 Page 118).



Graph 41: The **RMI** Point of Response, in days, for seven **CML** allo**PBSCT** patients, 50%, 75% and 95% are highlighted.

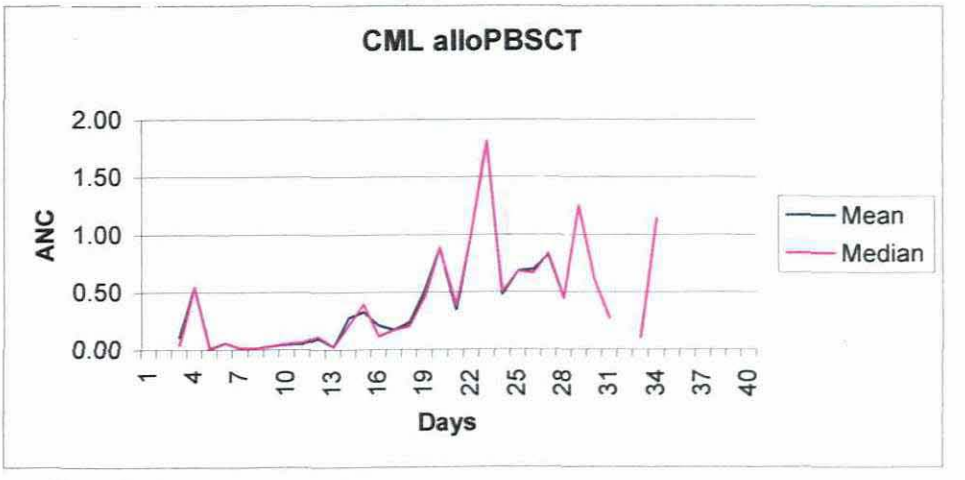
One hundred percent of the patients responded within 95% Point of Response.

The sixteen day Point of Response indicated nothing new compared to the other groups analysed.

4.7.2. The Absolute Neutrophil Count (ANC) Response

4.7.2.1. The Mean and Median Response Time for the Absolute Neutrophil Count (ANC) in Chronic Myeloid Leukaemia allogenic Peripheral Blood Stem Cell Transplant (CML alloPBSCT) Patients

The mean Response Time was twenty days, with a range of fifteen to twenty nine days (Figure 5 Page 39). The median Response Time was twenty one days, with a range of fifteen to twenty nine days (Figure 6 Page 40). All the patients responded within twenty nine days.



Graph 42: The mean and median **ANC** Response Time, in days for seven **CML** allo**PBSCT** patients.

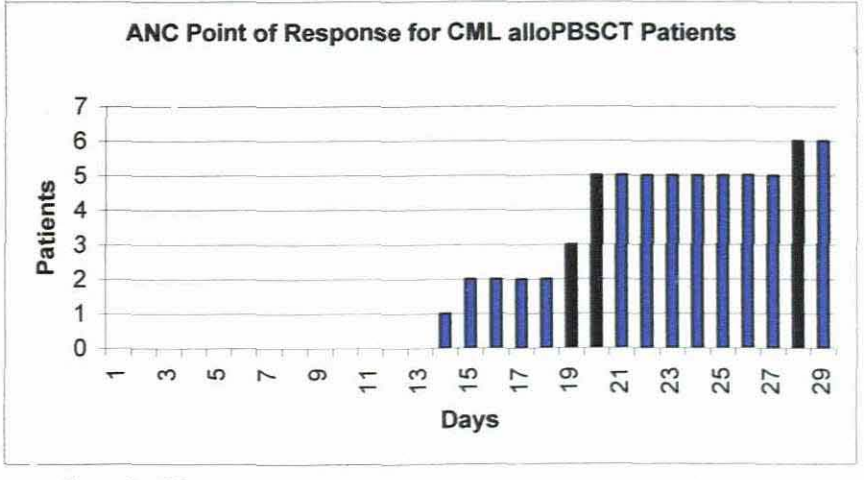
A **CML** allo**PBSCT** mean **ANC** 0.12 $x10^9$/l was seen within thirty six days (Graph 42 Page 119), 1.69 $x10^9$/l at three months, 1.87 $x10^9$/l at four months, 1.56 $x10^9$/l at five months and 2.52 $x10^9$/l at six months, a two thousand percent increase (Figure 12 Page 47). A median of 0.12 $x10^9$/l was seen within thirty six days (Graph 42 Page 119), 0.92 $x10^9$/l at three months, 0.94 $x10^9$/l at four months, 1.38 $x10^9$/l at five months and 2.85 $x10^9$/ at six months, a two thousand two hundred and seventy five percent increase (Figure 13 Page 47).

The **RMI** mean Response Time was nine days earlier than the **ANC** response indicating earlier erythroid recovery.

The **ANC** mean Response Time was the slowest of all the groups, at twenty days. The group also produced the lowest mean count at thirty six days of 0.12 $x10^9$/l, followed by a phenomenal mean increase of two thousand percent to 2.52 $x10^9$/l within six months. The incredibly high increase could be directly linked to the seventy one percent death rate. **CML** usually have the highest leukocyte counts on admission, with the possibility of relapse, this could be the cause of the increased **ANC** over six months.

4.7.2.2. The Point of Response for the Absolute Neutrophil Count (ANC) in Chronic Myeloid Leukaemia allogenic Peripheral Blood Stem Cell Transplant (CML alloPBSCT) Patients

A 95% Point of Response was seen within twenty eight days, 75% within twenty days and 50% within nineteen days (Graph 43 Page 120).



Graph 43: The **ANC** Point of Response, in days, for seven **CML** allo**PBSCT** patients, 50%, 75% and 95% are highlighted.

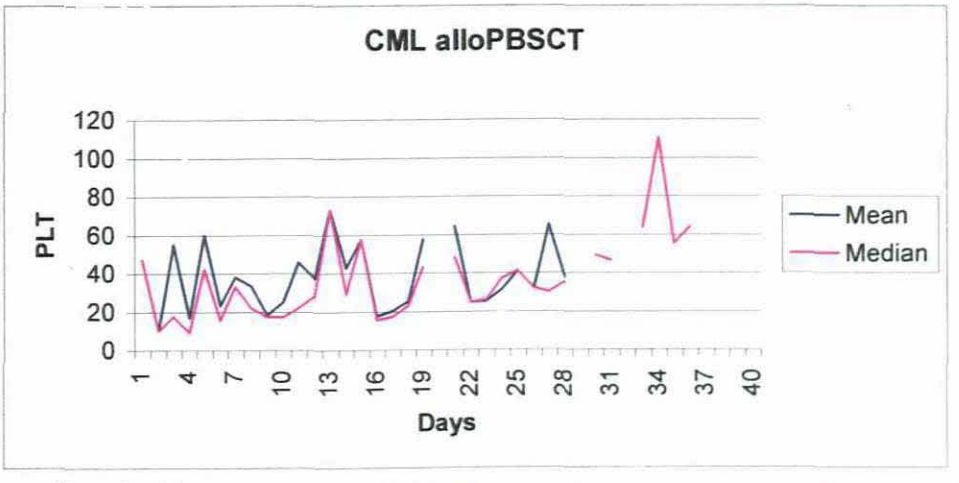
One hundred percent of the patients responded within 95% Point of Response. Even with the longest **ANC** mean Response Time, the Point of Response was medial. The **RMI** Point of Response was twelve days prior to the **ANC** response indicating earlier erythroid recovery.

The **ANC** Point of Response was twenty eight days. Only one patient responded on day forty three, she was considered a "delayed response". The patient was classified a "delayed response" in all the groups and was not used in the calculation, even though she achieved a "12 month post transplant trilineage response".

4.7.3. The Platelet Count (PLT) Response

4.7.3.1. The Mean and Median Response Time for the Platelet Count (PLT) in Chronic Myeloid leukaemia allogenic Peripheral Blood Transplant (CML alloPBSCT) Patients

The mean (Figure 5 Page 39) and median (Figure 6 Page 40) Response Time was twenty one days, with a range of seven to thirty six. All the patients responded within thirty six days.



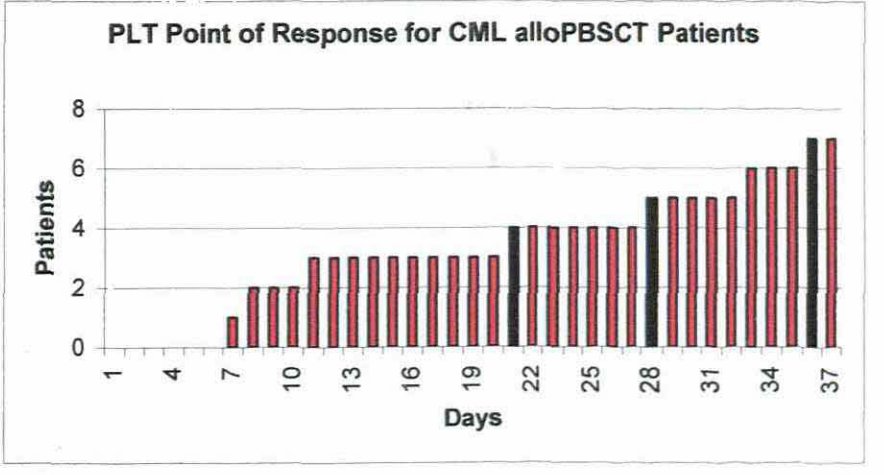
Graph 44: The mean and median **PLT** Response Time, in days, for seven **CML** allo**PBSCT** patients.

A **CML** allo**PBSCT** mean **PLT** of 64 $x10^9$/l was seen within thirty six days (Graph 44 Page 121), 87 $x10^9$/l at three months, 128 $x10^9$/l at four months, 140 $x10^9$/l at five months and 141 $x10^9$/l at six months, a mean increase of one hundred and twenty percent (Figure 14 Page 48). A median of 64 $x10^9$/l was seen within thirty six days (Graph 44 Page 121), 93 $x10^9$/l at three months, 141 $x10^9$/l at four months, 126 $x10^9$/l at five months and 161 $x10^9$/l at six months, a median increase of one hundred and fifty two percent (Figure 15 Page 48).

The **PLT** Response Time like the **ANC** was the slowest of all the groups. The **RMI** mean Response Time was nine and ten days earlier than the **ANC** and **PLT** response. This indicated an earlier erythroid recovery.

4.7.3.2. The Point of Response for the Platelet Count (PLT) in Chronic Myeloid Leukaemia allogenic Peripheral Blood Stem Cell Transplant (CML alloPBSCT) Patients

A 95% Point of Response was seen within thirty six days, 75% within twenty eight days and 50% twenty one days (Graph 45 Page 122).



Graph 45: The **PLT** Point of Response, in days, for seven **CML** allo**PBSCT** patients, 50%, 75% and 95% are highlighted.

One hundred percent of the patients responded within 95% Point of Response. The **ANC** "delayed response" patient responded within the range for both the **RMI** and the **PLT**. The **RMI** Point of Response was twenty days prior to the **PLT** where the **ANC** was only eight days prior. The erythroid recovery was the earliest. The erythroid line did not appear to be affected by the slow response phenomenon observed within the granulocytic and megakaryocytic lines.

4.7.4. Preconditioning in Chronic Myeloid Leukaemia allogenic Peripheral Blood Stem Cell Transplant (CML alloPBSCT) Patients

The preconditioning of the seven patients consisted of the following:

- Five **Cyclo/TBI/TNI.**
- Two **Bu/Cy.**

Seventy one percent of the patients were treated with **Cyclo/TBI/TNI**. Sixty percent of them produced an early **RMI** response. One patient achieved a "12 month post transplant trilineage response". Only one patient treated with **Cyclo/TBI/TNI** produced an early **ANC** response, however she died. There were two patients with an early **PLT** response, but both died. Although more than fifty percent of the patients generated early responses, indicating minimal stromal damage, very few of them projected a positive medium term prognosis.

Of the two patients who were treated with **Bu/Cy**, only one achieved a "12 month post transplant trilineage response". She was the only patient to produce an earlier than mean **PLT**. Although the trend for **CML** patients was to respond later, particularly in the **ANC** parameter, this however, possibly had nothing to do with the preconditioning. Patient numbers were too low accurate evaluation.

4.7.5. Mononuclear Count (MNC), Colony Forming Unit – Granulocytic Monocytic (CFU-GM) and Cluster Designation 34+ Counts (CD34+) in Chronic Myeloid Leukaemia allogenic Peripheral Blood Stem Cell Transplant (CML alloPBSCT) Patients

All the patients were evaluated for **MNC**, with a mean of 6.69 $x10^8$/kg (Figure 8 Page 44). Only forty three percent of them generated counts greater than the mean. One patient produced an early **RMI** response, however she died. No early **ANC** responses were found. Of the two early **PLT** responses, one produced a raised **MNC**; he achieved a "12 month post transplant trilineage response". Ultimately less than fifty percent of the patients generated early responses in all three blood parameters. Even with the low patient numbers, lower **MNC** possibly resulted in later peripheral blood responses, not to mention the poor projection of success on medium term success.

Only fifty seven percent of the patients were evaluated for **CFU-GM** counts, with a mean of 22.21 $x10^4$/kg. Seventy five percent of them produced counts higher than the group mean, only one achieved a successful "12 month post transplant trilineage response". This patient was a child, with the early **PLT** response. Patient numbers were however too low for accurate evaluation.

Only two patients were evaluated for **CD34+** counts, with a mean of 53.78. One patient generated counts higher than the group mean, however she relapsed. The patient numbers were too low for accurate evaluation.

4.7.6. Response Range for Chronic Myeloid Leukaemia allogenic Peripheral Blood Stem Cell Transplant (CML alloPBSCT) Patients

4.7.6.1. Reticulocyte Maturation Index (RMI)

Of the seven patients represented within the group, only one generated an **RMI** above **2SD** within the first forty days, however she relapsed. None of the patients produced counts below the mean. These findings indicated nothing significant.

4.7.6.2. Absolute Neutrophil Count (ANC)

None of the seven patients generated **ANC** that exceeded **2SD** for the first forty days. This was understandable due to the late response seen in **CML** patients. However, forty three percent of the patient's produced **ANC** below **2SD** within the first forty days, twenty nine percent of them died and fourteen percent achieved a "12 month post transplant trilineage response". The raised number of low **ANC** is possibly significant, as late responses could ultimately lead to poor recovery.

4.7.6.3. Platelet Count (PLT)

One hundred percent of the patients generated **PLT** greater than the mean within the first forty days. No patients had **PLT** fall to zero counts at any point.

4.7.7. "Day 14" Marrow Biopsy Results for Chronic Myeloid Leukaemia allogenic Peripheral Blood Stem Cell Transplant (CML alloPBSCT) Patients

Of the twenty nine percent who received "day 14" marrow biopsies, none of the patient biopsies indicated any additional information compared to the "day 28" marrow. For **CML** transplants, the suggestion would be to concentrate on the peripheral blood counts rather than "day 14" biopsies, particularly because of the later responses, particularly in the **ANC** and **PLT** parameters. The blood results appear be a more accurate means of engraftment verification.

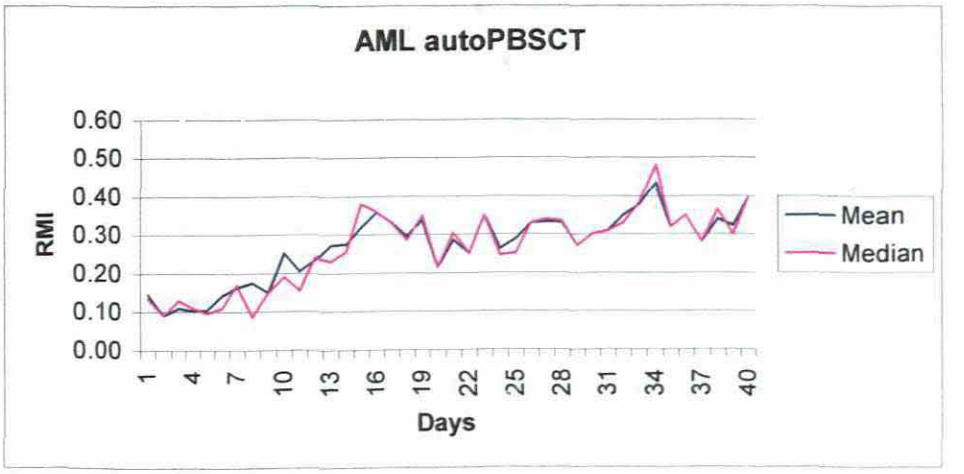
4.8. Acute Myeloid Leukaemia autologous Peripheral Blood Stem Cell Transplant (AML autoPBSCT) Patients

There were nine auto**PBSCT AML** patients analysed.

4.8.1. The Reticulocyte Maturation Index (RMI) Response

4.8.1.1. The Mean and Median Response Time for the Reticulocyte Maturation Index (RMI) in Acute Myeloid Leukaemia autologous Peripheral Blood Stem Cell Transplant (AML autoPBSCT) Patients

The mean (Figure 5 Page 39) and median (Figure 6 Page 40) Response Time was ten days, with a range of seven to fourteen days. All the patients responded within fourteen days.



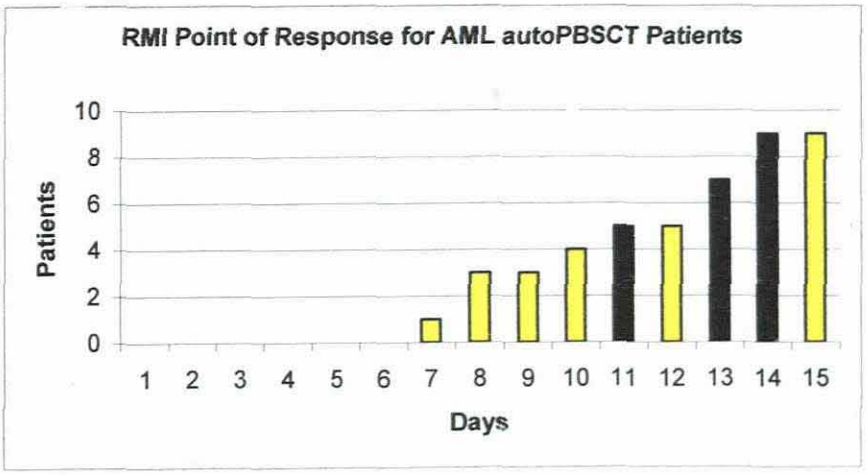
Graph 46: The mean and median **RMI** Response Time, in days, for nine **AML** auto**PBSCT** patients.

An **AML** auto**PBSCT** mean **RMI** of 0.40 IU was seen within forty days (Graph 46 Page 125), 0.29 IU at three months, 0.29 IU at four months, 0.21 IU at five months and 0.18 IU at six months, a one hundred and twenty two percent decrease (Figure 10 Page 46). A median of 0.40 IU was seen within forty days (Graph 46 Page 125), 0.33 IU at three months, 0.32 IU at four months, 0.21 IU at five months and 0.16 IU at six months, a one hundred and fifty percent decrease (Figure 11 Page 46).

Even with a ten day **RMI** mean Response Time, and a one hundred and twenty two percent decrease was recorded within six months, the **AML** auto**PBSCT** produced a sixty seven percent "12 month post transplant trilineage response".

4.8.1.2. The Point of Response for the Reticulocyte Maturation Index (RMI) in Acute Myeloid Leukaemia autologous Peripheral Blood Stem Cell Transplant (AML autoPBSCT) Patients

A 95% Point of Response was seen within fourteen days, 75% response within thirteen days and 50% response within eleven days (Graph 47 Page 126).



Graph 47: The **RMI** Point of Response, in days, for nine **AML** auto**PBSCT** patients, 50%, 75% and 95% are highlighted.

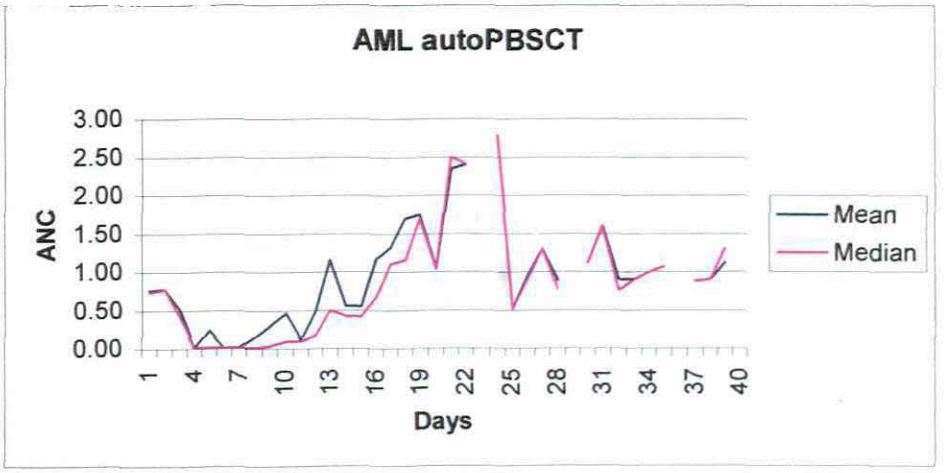
One hundred percent of the patients responded within 95% Point of Response.

Along with the "12 month post transplant trilineage response" group the **AML** auo**PBSCT** produced the earliest Point of Response at fourteen days. This was possibly due to the high success rate of the **AML** auto**PBSCT** in achieving a "12 month post transplant trilineage response".

4.8.2. The Absolute Neutrophil Count (ANC) Response

4.8.2.1. The Mean and Median Response Time for Absolute Neutrophil Count (ANC) in Acute Myeloid Leukaemia autologous Peripheral Blood Stem Cell Transplant (AML autoPBSCT) Patients

Both the mean (Figure 5 Page 39) and median (Figure 6 Page 40) Response Time was fifteen days, with a range of nine to twenty one days. All the patients responded within twenty one days.



Graph 48: The mean and median **ANC** Response Time, in days, for nine **AML** auto**PBSCT** patients.

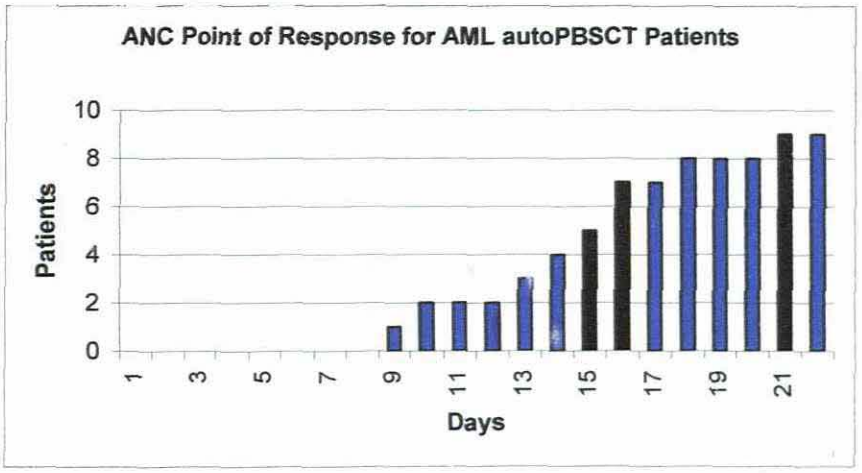
An **AML** auto**PBSCT** mean **ANC** of 1.12 x10^9/l was seen within thirty nine days (Graph 48 Page 127), 2.09 x10^9/l at three months, 2.70 x10^9/l at four months, 1.71 x10^9/l at five months and 2.91 x10^9/l at six months, a one hundred and sixty percent increase (Figure 1 Page 47). The median of 1.30 x10^9/l was seen by day thirty nine (Graph 48 Page 127), 1.28 x10^9/l at three months, 1.71 x10^9/l at four months, 1.78 x10^9/l at five months and 2.78 at six months, a one hundred and fourteen percent increase (Figure 13 Page 47).

The mean **RMI** Response Time was five days earlier than the **ANC** response. This indicated an earlier erythroid recovery.

Both the auto**PBSCT** group and the **AML** auto**PBSCT** group produced **ANC** responses within two and three days of the **RMI** response. These finding were possibly due to the "pattern of recognition" seen within the autologous transplants.

4.8.2.2. The Point of Response for the Absolute Neutrophil Count (ANC) in Acute Myeloid Leukaemia autologous Peripheral Blood Stem Cell Transplant (AML autoPBSCT) Patients

A 95% Point of Response was seen within twenty one days, 75% within sixteen days and 50% within fifteen days (Graph 49 Page 128).



Graph 49: The **ANC** Point of Response, in days, for nine **AML** auto**PBSCT** patients, 50%, 75% and 95% are highlighted.

One hundred percent of the patients responded within 95% Point of Response.

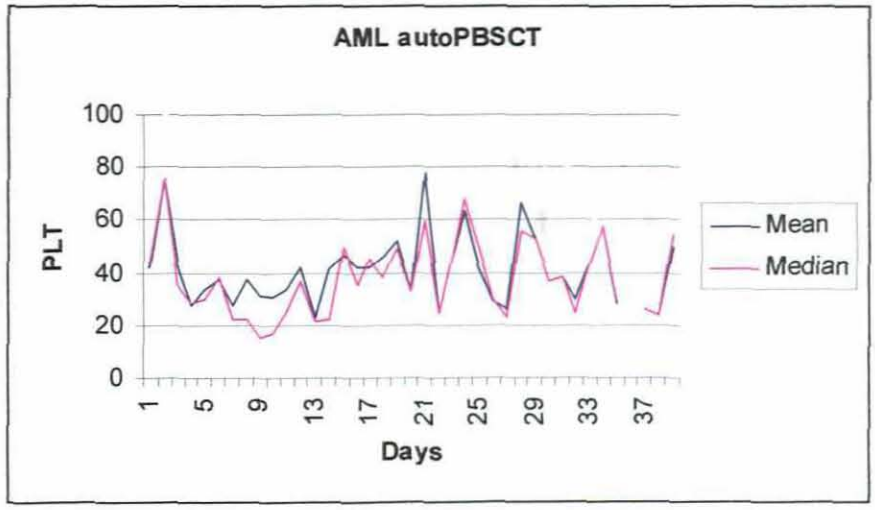
The **RMI** Point of Response was six days prior to the **ANC** response indicating earlier erythroid recovery.

The **ANC** Point of Response was the same as the autologous transplants, namely twenty one days. All the autologous transplants generated early responses; counts above 0.40 $x10^9/l$ at forty days, and a percentage increase within six months in excess of one hundred percent. The autologous transplants clearly feature the earliest responses and the highest medium term success rate of seventy one percent

4.8.3. The Platelet Count (PLT) Response

4.8.3.1. The Mean and Median Response Time for the Platelet Count (PLT) in Acute Myeloid Leukaemia autologous Peripheral Blood Stem Cell Transplant (AML autoPBSCT) Patients

The mean Response Time was eighteen days, with a range of eleven to twenty eight days (Figure 5 Page 39). The median Response Time was nineteen days, with a range of eleven to twenty eight days (Figure 6 Page 40). All the patients responded within twenty eight days.



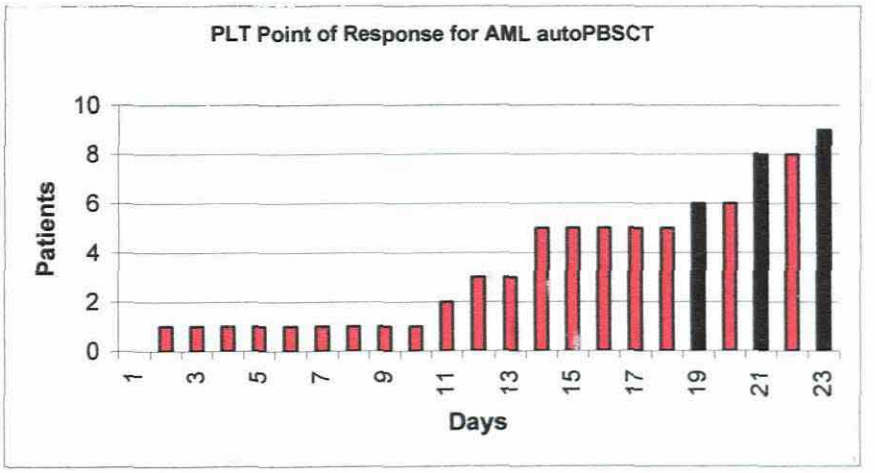
Graph 50: The mean and median **PLT** Response Time, in days, for nine **AML** auto**PBSCT** patients.

An **AML** auto**PBSCT** mean **PLT** of 49 x10^9/l was seen within thirty nine days (Graph 50 Page 129), 123 x10^9/l at three months, 152 x10^9/l at four months, 111 x10^9/l at five months and 133 x10^9/l at six months, a mean increase of one hundred and seventy one percent (Figure 14 Page 48). A median of 54 x10^9/l was seen within thirty nine days (Graph 50 Page 129), 147 x10^9/l at three months, 178 x10^9/l at four months, 115 x10^9/l at five months and 163 x10^9/l at six months, a three hundred and fifty three percent increase (Figure 15 Page 48).

The mean **RMI** Response Time was eight days prior to the **PLT** response and three days earlier than the **ANC** response. The erythroid recovery was the earliest. The **PLT** mean Response Time was one of the slowest of all the groups analysed.

4.8.3.2. The Point of Response for the Platelet Count (PLT) in Acute Myeloid Leukaemia autologous Peripheral Blood Stem Cell Transplant (AML autoPBSCT) Patients

A 95% Point of Response was seen within twenty three days, 75% within twenty one days and 50% within nineteen days (Graph 51 Page 130).



Graph 51: The **PLT** Point of Response for nine **AML** auto**PBSCT** patients, in days, 50%, 75% and 95% highlighted.

One hundred percent of the patients responded within 95% Point of Response.

The **RMI** Point of Response was ten days prior to the **PLT** response and six days prior to the **ANC** response. The erythroid recovery was the earliest.

The **PLT** Point of Response was the earliest of all the groups analysed, at twenty three days. The only other groups to produce responses earlier than thirty days were the autologous transplant group and the auto**PBSCT** group. They also generated slow mean Response Times. The forty day **PLT** counts for the **AML** auto**PBSCT** were of the lowest, and the percentage increase for the six month period was not of the highest. Although, all three of the autologous transplant groups produced a greater than one hundred percent increase within six months. Like the **ANC** early responses, an early

PLT response appeared to indicate a recipe for a "12 month post transplant trilineage response".

4.8.4. Preconditioning in Acute Myeloid autologous Peripheral Blood Stem Cell Transplant (AML autoPBSCT) Patients

All nine patients received preconditioning; treatment was as follows:

- Four **Bu/Cy.**
- Four **Cyclo/TBI/TNI.**
- One **Bu/Mel/Thiotep.**

Sixty seven percent of the patients preconditioned achieved a "12 month post transplant trilineage response". Of the forty four percent treated with **Cyclo/TBI/TNI**, fifty percent (two) of them produced an early **RMI** response; while both achieved a " 12 month post transplant trilineage response". Of the forty four percent treated with **Bu/Cy**, seventy five percent of them achieved a "12 month post transplant trilineage response" only twenty five percent of them produced an early **RMI** response. Of the thirty three percent who produced an early **ANC** response, two were treated with **Bu/Cy**, one achieved a "12 month post transplant trilineage response", the third was treated with **Cyclo/TBI/TNI**, and he achieved a "12 month post transplant trilineage response". The patient, who was treated with **Bu/Mel/Thiotep**, generated early **RMI** and **ANC** responses, however she died. Forty four percent of the patients generated early **PLT** responses, one treated with **Bu/Cy** and two treated with **Cyclo/TBI/TNI** achieved a "12 month post transplant trilineage response". Unfortunately, patient numbers were too low, and the early responses were too varied for accurate assessment.

4.8.5. Mononuclear Count (MNC), Colony Forming Unit - Granulocytic Monocytic (CFU-GM) and Cluster Designation 34+ Counts (CD34+) in Acute Myeloid Leukaemia autologous Peripheral Blood Stem Cell Transplant (AML autoPBSCT) Patients

All the patients were evaluated for **MNC**, the mean was 5.97 $x10/^{8}$/kg. Only sixty seven percent of the patients were evaluated for **CFU-GM** counts, the mean was 27.84 $x10^{4}$/kg; thirty three percent of the patients produced in no growth, due to bacterial contamination. Only twenty two percent of the patients were evaluated for **CD34+** counts, the mean was 137.55 (Figure 8 Page 44).

Twenty two percent (two) of the patients who generated early **RMI** responses, produced higher **MNC**, both achieved a "12 month post transplant trilineage response". Forty four percent (four) of the patients failed to generate early **RMI** responses or higher **MNC**, two achieved a "12 month post transplant trilineage response". None of the patients with higher **MNC** generated early **ANC** responses; one produced an early **PLT** response. Although the **RMI** appeared to be the only parameter to experience early responses, the patients with higher **MNC** achieved a "12 month post transplant trilineage response". The patient numbers were ultimately too low for accurate assessment.

Only fifty five percent of the patients generated **CFU-GM** counts, only one produced a count greater than the mean. He generated early **RMI** and **PLT** responses and achieved a "12 month post transplant trilineage response". Unfortunately, being the only patient with early responses, no accurate assessment could be made.

Only twenty two percent (two) of the patients had **CD34+** counts performed. There was a vast difference in counts, 27,82 and 247.28. No accurate assessment could be made.

4.8.6. Response Range for Acute Myeloid Leukaemia autologous Peripheral Blood Stem Cell Transplant (AML autoPBSCT) Patients

4.8.6.1. Reticulocyte Maturation Index (RMI)

None of the patients produced **RMI** counts above or below **2SD** within the first forty days. Unfortunately in the case of **AML** auto**PBSCT**, raised or low **RMI** counts appear to show no prediction value.

4.8.6.2. Absolute Neutrophil Count (ANC)

Of all the patients with **ANC** above **2SD** within the first forty days, thirty three percent of them were **AML** auto**PBSCT.** Sixty seven percent achieved a "12 month post transplant trilineage response", one patient died from unrelated causes. Twenty two percent of the patients fell below **2SD**; both failed to achieve a "12 month post transplant trilineage response". Although shown those higher counts without clinical infection indicates successful transplants, it is surprising that only a thirty three percent of the patients generated counts above **2SD**.

4.8.6.3. Platelet Count (PLT)

Within five days, one hundred percent of the patients' achieved a **PLT** higher than **2SD**. None of the patients **PLT** dropped to zero at any point. The high **PLT** did not reveal any information regarding response prediction.

4.8.7. "Day 14" Marrow Biopsy Results on Acute Myeloid Leukaemia autologous Peripheral Blood Stem Cell Transplant (AML autoPBSCT) Patients

"Day 14" marrow biopsies were performed on twenty two percent of the **AML** auto**PBSCT**. These biopsies revealed no additional information to the "day 28" biopsies.

"Day 14" marrow biopsies appear to be an irrelevant procedure, provided that peripheral blood parameters are monitored on a regular basis together with the "day 28" marrow biopsy.

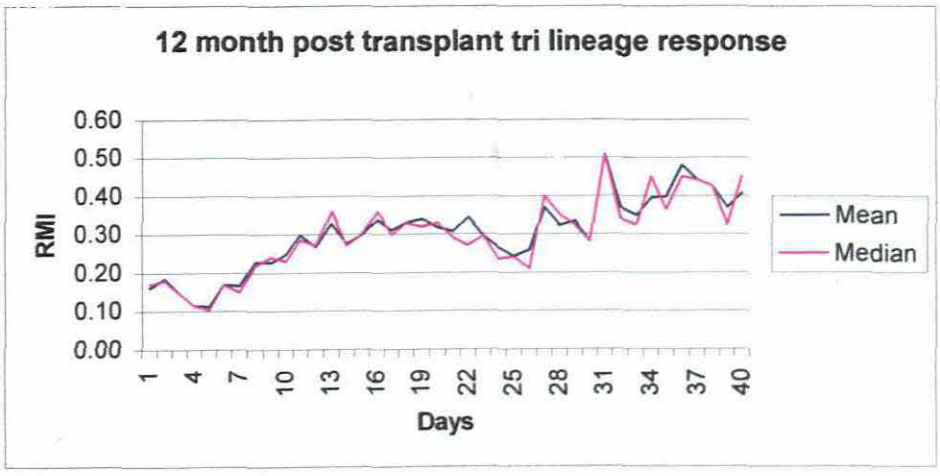
4.9. "12 month post transplant trilineage response"

Twenty two "12 month post transplant trilineage response" patients were analysed.

4.9.1. The Reticulocyte Maturation Index (RMI) Response

4.9.1.1. The Mean and Median Response Time for the Reticulocyte Maturation Index (RMI) in "12 month post transplant trilineage response" Patients

The mean (Figure 5 Page 39) and median (Figure 6 Page 40) Response Time was eight days, with a range of one to seventeen days. All the patients responded within seventeen days.



Graph 52: The mean and median **RMI** Response Time, in days, for twenty two "12 month post transplant trilineage response" patients.

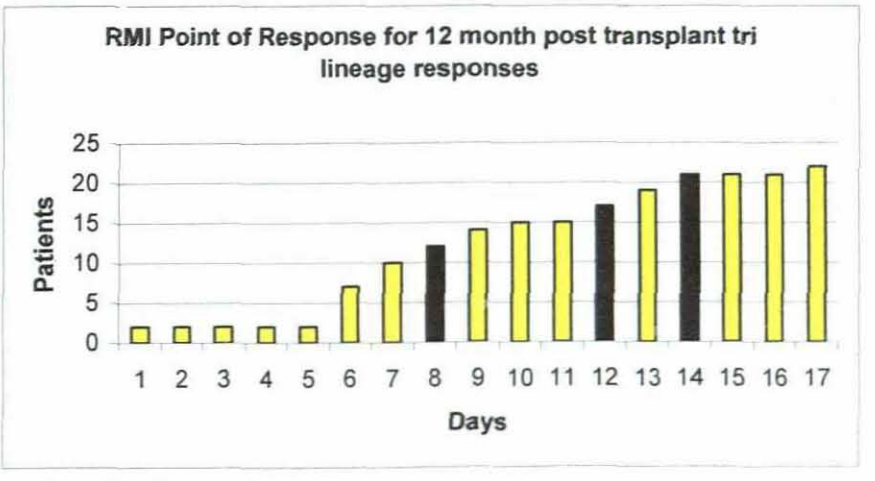
A mean **RMI** of 0.41 IU was seen within thirty nine days (Graph 52 Page 134), 0.29 IU at three months, 0.25 IU at four months, 0.20 IU at five months and 0.17 IU at six months, a mean decrease of one hundred and forty one percent (Figure 10 Page 46). A median of 0.45 IU was seen within thirty nine days (Graph 52 Page 134), 0.30 IU at three months, 0.23 IU at four months, 0.19 IU at five months and 0.16 IU at six months, a median decrease of one hundred and eighty one percent (Figure 11 Page 46).

The "12 month post transplant trilineage response" group produced a mean **RMI** Response Time of eight days, and a one hundred and forty one percent decrease within six months. They produced the second highest decrease of the eleven groups analysed, second to the auto**PBSCT** group at a sixty seven percent decrease. A decrease in **RMI**

values over six months did not appear to have any negative effects on medium term success, because of the hundred percent success rate. With an initial anaemia post transplant, the erythrocyte component was the first to recover, resulting in an active correction of anaemia. The counts levelled out, resulting in a slightly below normal **RMI** at six months. Further studies would need to be done to evaluate the long term effect of **RMI** values on long term successful transplants.

4.9.1.2. The Point of Response for the Reticulocyte Maturation Index (RMI) in "12 month post transplant trilineage response" Patients

A 95% Point of Response was seen within fourteen days, 75% within twelve days and 50% within eight days (Graph 53 Page 135).



Graph 53: The **RMI** Point of Response, in days, for twenty two "12 month post transplant trilineage response" patients, 50%, 75% and 95% are highlighted.

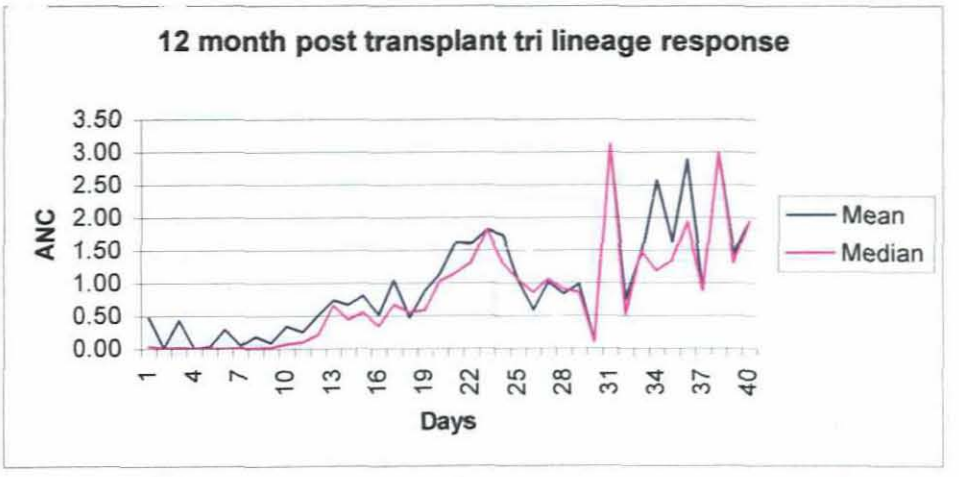
Ninety five percent of the patients responded within 95% Point of Response. One patient, a **NHL** auto**PBSCT**, responded on day seventeen, he was not considered a "delayed response".

This group along with the **AML** auto**PBSCT** were the only two to generate a fourteen day Point of Response. Considering the excellent medium term success rate seen in both, it was understandable.

4.9.2. The Absolute Neutrophil Count (ANC) Response

4.9.2.1. The Mean and Median Response Time for the Absolute Neutrophil Count (ANC) in "12 month post transplant trilineage response" Patients

The mean Response Time was sixteen days (Figure 5 Page 39), and the median Response Time was fourteen days (Figure 6 Page 40), with a range of six to thirty two days. All the patients responded within thirty two days.



Graph 54: The mean and median **ANC** Response Time, in days, for twenty two "12 month post transplant trilineage response" patients.

A mean **ANC** of 1.94 $x10^9/l$ was seen within thirty nine days (Graph 54 Page 136), 2.25 $x10^9/l$ at three months, 2.27 $x10^9/l$ at four months, 2.18 $x10^9/l$ at five months and 2.92 $x10^9/l$ at six months, a fifty one percent increase (Figure 12 Page 47). A median of 1.94 $x10^9/l$ was seen within thirty nine days (Graph 54 Page 136), 1.76 $x10^9/l$ at three months, 1.90 $x10^9/l$ at four months, 1.78 $x10^9/l$ at five months and 2.28 $x10^9/l$ at six months, an eighteen percent increase (Figure 13 Page 47).

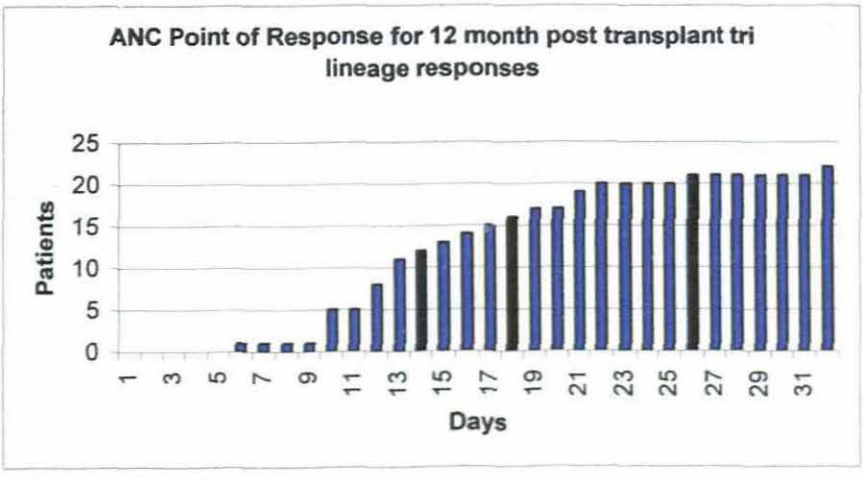
The **RMI** mean Response Time was eight days earlier than the **ANC** response. The erythroid recovery was the earlier of the two.

The **ANC** mean Response Time was sixteen days, and a fifty one percent increase was seen within six months. Both the mean **ANC** at thirty nine days and the percentage increase over six months were the second highest of all the groups, second to the **BMT**. The outcome was an indication that a healthy **ANC** response within the first forty days is

important. If a patient were to achieve a healthy count within sixteen days an improved chance of medium term success would be possible. The "12 month post transplant trilineage response" group was the only group analysed to produce an improved monthly **ANC.** This factor could be to the advantage for transplant patients, particularly if their **ANC** continued to increase under normal conditions. Although the rise in **ANC** over six months paralleled the **RMI** decrease in successful transplant patients, this was possibly of little value.

4.9.2.2. The Point of Response for the Absolute Neutrophil Count (ANC) in "12 month post transplant trilineage response" Patients

A 95% Point of Response was seen within twenty six days, 75% within eighteen days and 50% within fourteen days (Graph 55 Page 137).



Graph 55: The **ANC** Point of Response, in days, for twenty two "12 month post transplant trilineage response" patients, 50%, 75% and95% are highlighted.

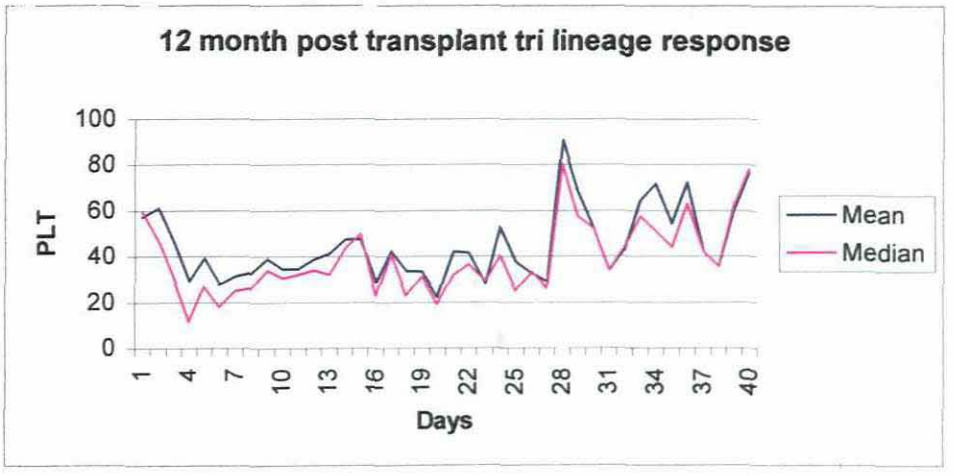
Ninety five percent of the patients responded within 95% Point of Response. One patient, a **NHL BMT** who responded on day thirty two, was considered a "slightly delayed" response.

The **RMI** Point of Response was seen twelve days prior to the **ANC** response. The erythroid engraftment was the earlier of the two.

4.9.3. The Platelet Count (PLT) Response

4.9.3.1. The Mean and Median Response Time for the Platelet Count (PLT) in "12 month post transplant trilineage response" Patients

The mean Response Time was sixteen days (Figure 5 Page 39) and median fourteen days (Figure 6 Page 40) with a range of one to thirty six days. All the patients responded within thirty six days.



Graph 56: The mean and median **PLT** Response Time, in days, for twenty two "12 month post transplant trilineage response" patients.

A mean **PLT** of 76 $x10^9$/l was seen within thirty nine days (Graph 56 Page 138), 91 $x10^9$/l at three months, 120 $x10^9$/l at four months, 138 $x10^9$/l at five months and 164 $x10^9$/l at six months, a mean increase of one hundred and sixteen percent (Figure 15 Page 48). A median of 78 $x10^9$/l was seen within thirty nine days (Graph 56 Page 138), 93 $x10^9$/l at three months, 102 $x10^9$/l at four months, 119 $x10^9$/l at five months and 132 $x10^9$/l at six months, a median increase of sixty nine percent (Figure 13 Page 48).

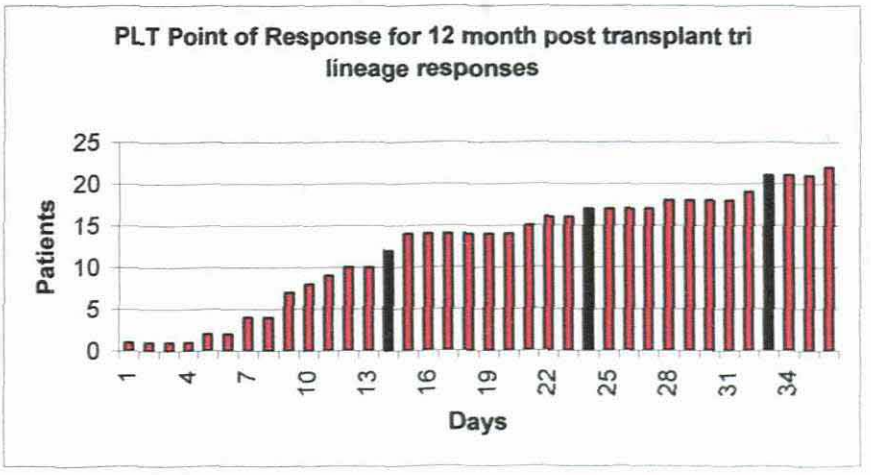
The mean **RMI** Response Time was seven days prior to the **PLT** response and the **ANC** was one day earlier. The erythroid engraftment was the earliest of the three.

There was a considerable difference between the **PLT** mean and median Response Time percentage over six months, at one hundred and sixteen and sixty nine percent respectively. This was possibly due to the **CML** allo**PBSCT** patient with low counts.

The mean **PLT** response Time was twenty four days, at one hundred and sixteen percent increase over six months. The median counts were more informative. With a thirty nine day median **PLT** count of 78 $x10^9$/l, to a count of 132 $x10^9$/l within six months. It appeared that for a positive medium term success, a healthy **PLT** response should be seen within the first forty days. Healthy forty day counts should be achieved regardless of the percentage increase over a six month period.

4.9.3.2. The Point of Response for the Platelet Count (PLT) in "12 month post transplant trilineage response" Patients

A 95% Point of Response was seen within thirty three days, 75% within twenty four days and 50% within fourteen days (Graph 57 Page 139).



Graph 57: The **PLT** Point of Response, in days for twenty two "12 month post transplant trilineage response" patients, 50%, 75% and 95% are highlighted.

Ninety five percent of the patients responded within 95% Point of Response. One patient a **CML** allo**PBSCT** responded on day thirty six; she was considered a "slightly delayed response".

The **RMI** Point of Response was seen nineteen days prior to the **PLT** response and the **ANC** was seven days earlier. The erythroid engraftment was the earliest of the three.

The **PLT** Point of Response was similar to most of the groups analysed, and not as significant as the **RMI** or **ANC** response. More information was gathered from the median Response Time, as mentioned previously (Page 139).

4.9.4. Preconditioning in "12 month post transplant trilineage response" Patients

Of the twenty two patients receiving preconditioning, the treatment was as follows:

- Thirteen **Cyclo/TBI/TNI** - five **BMT** and eight **PBSCT.**
- Five **Bu/Cy** patients - All **PBSCT,** one **CML** allo**PBSCT**, one stem cell leukaemia auoto**PBSCT** and three **AML** auto**PBSCT**.
- Two **Bu/Mel/Thiotep** - Both **NHL** auto**PBSCT** patients.
- Two **Cyclo/TNI** – Both **SAA** allo**PBSCT** patien.s.

Fifty nine percent of the patients were treated with **Cyclo/TBI/TNI.** One hundred percent of the **BMT** received **Cyclo/TBI/TNI**. Sixty percent of them produced an early **RMI** response, forty percent an early **ANC** response and sixty percent an early **PLT** response. Forty seven percent of the patients treated with **Cyclo/TBI/TNI** were **PBSCT.** Seventy five percent of them produced an early **RMI** response, sixty three percent an early **ANC** response, and seventy five percent an early **PLT** response. In both **BMT** and **PBSCT**, higher percentages of early **RMI** and **PLT** responses were seen compared to the **ANC**. This was possibly due to the **CML** allo**PBSCT** with a "delayed **ANC** response".

Of the thirty eight percent treated with **Bu/Cy**, only one patient produced an earlier **RMI** response, none an early **ANC** response, but sixty percent produced an early **PLT** response. Even with the low patient numbers the preconditioning had the lowest effect on the megakaryocytic line.

One of the patients treated with **Bu/Mel/Thiotep** generated early responses in all three blood parameters. The other, an early response was seen only in the **PLT**. Patient numbers were too low for an accurate assessment.

Both of the successful **SAA** allo**PBSCT's** received the **Cyclo/TNI** preconditioning regime. One patient generated an early response in all three blood parameters, the other early responses were seen in the **RMI** and **PLT** parameter. The patient numbers were too low for an accurate assessment.

4.9.5. Mononuclear Count (MNC), Colony Forming Unit – Granulocytic Monocytic (CFU-GM) and Cluster Designation 34+ Counts (CD34+) in "12 month post transplant trilineage response" Patients

MNC were performed on all of the patients, with a mean of 5.83 $x10^8$/kg. Only seventy three percent of the patients had **CFU-GM** counts, with a mean of 19.84 $x10^4$/kg, fourteen percent produced no growth. **CD34+** counts were performed on fourteen percent of the patients, with a mean of 113.68 (Figure 8 Page 44).

The **BMT** resulted in a mean **MNC** of 1.22 $x10^8$/kg. Only two patients generated early **RMI** and **ANC** responses, only one patient produced an early **PLT** response. Unfortunately, the results indicated nothing. The **PBSCT** resulted in a mean **MNC** of 7.18 $x10^8$/kg. Only forty one percent of these patients generated a count above the mean. Fifty seven percent of them generated early **RMI**, **ANC** and **PLT** response. Although more than fifty percent of the patients responded early in all three parameters, a higher percentage of patients with early responses would have been expected, particularly due to the fact that there was a one hundred percent medium term success rate. These results indicated that higher **MNC** can produce early responses, however medium term success is not always reliant on that fact.

Eighty percent (two patients) of the **BMT** had **CFU-GM** counts performed, with a mean of 19.59 $x10^4$/kg. Only fifty percent of them produced a count above the mean. Both of the patients produced an early **RMI** response, one an early **ANC** response. None of the patients produced an early **PLT** response. Seventy one percent of the **PBSCT** had **CFU-GM** counts performed. Only twenty five percent of them generated a count above the mean. Two of the three patients generated early **RMI**, **ANC** and **PLT** responses. Neither the **BMT** nor **PBSCT** produced sufficient patient numbers, to indicate that higher

CFU-GM counts produce earlier blood parameter responses, or that higher **CFU-GM** counts indicate a positive medium term prognosis.

Only fourteen percent (three patients) of the patients had **CD34+** counts, all were **PBSCT**, with a mean of 113.68. Although two of the patients produced counts below the mean, all three generated early **RMI** and **PLT** responses. The patient numbers were too low for an accurate assessment.

4.9.6. Response Range for "12 month post transplant trilineage response" Patients

4.9.6.1. Reticulocyte Maturation Index (RMI)

Only one patient generated **RMI** results above **2SD** within the first forty days. Two had had **RMI** values below **2SD**. Due to the low patient numbers an accurate assessment could not be made.

4.9.6.2. Absolute Neutrophil Count (ANC)

Thirty six percent of the patients had **ANC** counts above **2SD** within the first forty days. This supported the theory that higher counts without clinical infection indicated a positive prognosis for medium term success.

4.9.6.3. Platelet Count (PLT)

By day five, forty one percent of the patients had **PLT** counts above **2SD**; they were all **AML** auto**PBSCT**. The remainder of the patients had counts above **2SD** within forty days. None of the patients had **PLT** counts fall to zero.

4.9.7. "Day 14" Marrow Biopsy Results on "12 month post transplant trilineage response" Patients

Only twenty two percent of the patients received "day 14" marrow biopsies. These biopsies revealed no additional information to the "day 28" marrow biopsies. The "day 14" marrow biopsy appeared to be a procedure which was not entirely necessary. If peripheral blood counts were monitored on a regular basis, the "day 28" marrow would be sufficient. Particularly when the **RMI**, **ANC** and **PLT** are taken into account.

4.10. The Graft Failures

Four of the transplant patients failed to engraft. According to our research they were considered "graft failures". Their results were not used in any of the calculations. They were however, analysed individually. There were two **SAA** allo**BMT** patients and an **AML** auto**PBSCT** who failed to engraft, and all three died. The fourth patient was an **SAA** allo**PBSCT** patient, who failed to engraft after the first transplant. He achieved a "12 month post transplant trilineage response" following a booster (infusion of harvested cells without preconditioning).

4.10.1. The First Severe Aplastic Anaemia allogenic Bone Marrow Transplant (SAA alloBMT)

4.10.1.1. Reticulocyte Maturation Index (RMI) Response Time

Following the allo**BMT**, a **RMI** Response Time of eight days was recorded. These counts remained steady for twenty eight days. The **RMI** value then decreased below 0.20 IU, where it remained low until day forty two. An allo**PBSCT** was performed. The **RMI** only responded after twenty six days. The counts fluctuated for forty one days, until a count of 0.68 IU was finally achieved. No further peripheral blood results were available for analysis. The patient died within six months post transplant.

4.10.1.2. Reticulocyte Maturation Index (RMI) Point of Response

The **BMT** group **RMI** Point of Response was fifteen days. The patient's Point of Response was eight days following the allo**BMT**; his response fell within the 95% limit. Although he indicated an erythroid recovery, it was short lived; a count of 0.14 IU was recorded at forty two days. Following the allo**PBSCT**, no evidence of erythroid recovery was seen within the first seventeen days. According to our research this indicated an erythrocytic "graft failure".

4.10.1.3. Absolute Neutrophil Count (ANC) Response Time

No **ANC** response was seen after the allo**BMT**. An **ANC** response was seen within twenty five days following the allo**PBSCT**. These counts increased steadily reaching 4.57 $x10^9/l$ at forty days. No further peripheral blood results were available before he died.

4.10.1.4. Absolute Neutrophil Count (ANC) Point of Response

The **BMT** group **ANC** Point of Response was thirty two days. The patient failed to achieve a Point of Response within thirty six days after the allo**BMT.** According to our research this indicated a granulocytic "graft failure". The allo**PBSCT** Point of Response was twenty nine days. The patient responded within twenty five days following the allo**PBSCT**, with a count of 4.57 $x10^9$/l. The patient's count was well above the group mean (1.13 $x10^9$/l). Although the **ANC** indicated evidence of "graft failure" following the allo**BMT**, the **RMI** indicated "graft failure" following the allo**PBSCT**. This fact reiterates the importance that both parameters be used in monitoring recovery process post transplant.

4.10.1.5. Platelet Count (PLT) Response Time

The patient's **PLT** Response Time was nineteen days after the allo**BMT**. The counts were unstable prior to nineteen days, and fluctuated above and below 50 $x10^9$/l. The **PLT** fell below 10 $x10^9$/l within thirty eight days. **PLT** fluctuations above and below 50 $x10^9$/l were recorded during the forty seven days following the allo**PBSCT**.

4.10.1.6. Platelet Count (PLT) Point of Response

The **BMT** group **PLT** Point of Response was thirty three days. The patient's Point of Response was twenty one days. Although the patient responded within the 95% limit, fluctuations were evident the entire time. The **PLT** as a rule is not a clear indicator of engraftment. In this case no single specific point should be used to estimate **PLT** recovery or megakaryocytic "graft failure".

4.10.1.7. Preconditioning

Cyclo/TNI was the preconditioning prior to the allo**BMT**. **Cyclo/Alg** was the preconditioning prior to the allo**PBSCT**. Because of early **RMI** and **PLT** responses generated following the allo**BMT** and the early **ANC** and **PLT** responses generated following the allo**PBSCT**, very little stromal damage was indicated post infusion in either transplant.

4.10.1.8. Mononuclear Count (MNC) and Colony Forming Unit (CFU) Count

The **BMT** group mean **MNC** was 0.97 $x10^8$/kg, and the allo**PBSCT** mean was 7.93 $x10^8$/kg. The patient produced a **MNC** count of 1.01 $x10^8$/kg prior to the allo**BMT** and a count of 0.28 $x10^8$/kg prior to the allo**PBSCT**. Out research indicated that in **BMT** less than twenty five percent of patients with raised **MNC** generated early blood parameter responses, where allo**PBSCT** produced higher responses in more than fifty percent of the patients. Unfortunately the patient did not fit this criteria.

The **BMT** group mean **CFU-GM** was 16.75 $x10^4$/kg, and the allo**PBSCT** mean was 31.25 $x10^4$/kg. The patient produced a **CFU-GM** count of 4.70 $x10^4$/kg prior to the allo**BMT** and 3.28 $x10^4$/kg prior to allo**PBSCT**. In both transplants the patient's counts were well below the mean. Our research implied that in less than thirty percent of **BMT** patients with higher **CFU-GM** counts generated earlier parameter responses, the allo**PBSCT** produced higher responses in less than forty five percent of the patients. An early **PLT** response however, was seen in more than seventy seven percent of the allo**PBSCT** patients. This is possibly why no clear evidence of "graft failure" was indicated in either transplant.

4.10.1.9. The Response Range

- **Reticulocyte Maturation Index (RMI)**

The patient's **RMI** values rose above the mean on numerous occasions during the first forty two days, following the allo**BMT**, but never above **2SD**. After the allo**PBSCT** the **RMI** values fell below **2SD** on a few occasions prior to day twenty six. However, values greater than the **2SD** were seen between day twenty six and day forty. Our research suggested that **RMI** values greater or less than **2SD** within the first forty days post transplant were of little value when predicting post transplant recovery or medium term success. This patient's results were not contrary to the research.

- **Absolute Neutrophil Count (ANC)**

The patient's **ANC** constantly fell below the established mean from day ten following the allo**BMT**. After the allo**PBSCT** the **ANC** rose above the mean between twenty seven and forty days. Our research found that **ANC** greater than **2SD** within the first forty days, without any clinical infection, usually stood a good chance of achieving a "12

month post transplant trilineage response". This patient only produced **ANC** above the mean, with a poor outcome.

- **Platelet Count (PLT)**

The patient's **PLT** values ran below **2SD** for most of the forty two days following the allo**BMT**. After the allo**PBSCT, PLT** counts fluctuated above and below the mean for the first twenty seven days and then remained below the mean until day forty one. Our research revealed that **PLT** counts above or below **2SD** for the first forty days were of no value when predicting transplant recovery or medium term success. The patient's results were not contrary to these findings.

4.10.1.10. "Day 14" Marrow Biopsy

The patient's **BMT** indicated no engraftment in either the "day 14" or the "day 28" marrow biopsies. The allo**PBSCT** produced a trilineage response for the first three months. At six months, cellularity was seen to decrease, this continued until his death. The peripheral blood results indicated otherwise. The appearance of erythroid recovery and failure of granolocytic recovery was indicated post allo**BMT**. No erythrocytic recovery, but granulocytic recovery was indicated post allo**PBSCT**. Both transplants produced some megakaryocytic recovery.

4.10.2. The Second Severe Aplastic Anaemia allogenic Bone Marrow Transplant (SAA alloBMT)

4.10.2.1. Reticulocyte Maturation Index (RMI) Response Time

The patient's **RMI** Response Time was thirty seven days, eight days following the booster. At forty days the **RMI** was 0.43 IU, at three months 0.31 IU, at four months 0.20 IU, at five months 0.21 IU. A few days before her death the **RMI** values fell to 0.11 IU.

4.10.2.2. Reticulocyte Maturation Index (RMI) Point of Response

The **BMT** group **RMI** Point of Response was fifteen days. The patient's response was thirty seven days. According to our research, a thirty seven day response indicated a "graft failure". Following the booster the patient appeared to maintain a stable **RMI** for five months post transplant. Even with values exceptionally close to the mean, a steady

decrease ensued until she died. The booster did however, increase the **RMI** response, but failed to sustain life.

4.10.2.3. Absolute Neutrophil Count (ANC) Response Time

The patient's **ANC** Response Time was thirty four days, six days after the booster. She generated an **ANC** of 1.82 $x10^9/l$ within forty days, 0.37 $x10^9/l$ at three months, 0.01 $x10^9/l$ at four months. From five months the **ANC** remained less than 0.01 $x10^9/l$, until she died.

4.10.2.4. Absolute Neutrophil Count (ANC) Point of Response

The **BMT** group **ANC** Point of Response was thirty two days. The patient's response was thirty four days, six days following the boost. From day forty, although the **ANC** count was 1.04 $x10^9/l$, it was still below the mean (2.65 $x10^9/l$). This lower than average pattern continued for five months until no cells remained. Even with the initial questionable **ANC**, the delayed **RMI** value was a clear indicator of "graft failure". Both an **ANC** and **RMI** response was recorded following the booster. A rapid increase in both for a short period was evident, but not enough to sustain life. The **ANC** was seen to decrease more rapidly and dramatically than the **RMI.**

4.10.2.5. Platelet Count (PLT) Response Time

The patient's **PLT** Response Time was seventeen days. The count decreased within three days. The **PLT** fluctuated above and below 50 $x10^9/l$ continuously for five months. Even a booster failed to stabilise the **PLT**.

4.10.2.6. Platelet Count (PLT) Point of Response

The **BMT** group **PLT** Point of Response was thirty three days. Although the patient's **PLT** Point of Response was seventeen days, they remained unstable. Counts of 12 $x10^9/l$ were seen at forty days, 21 $x10^9/l$ at three months, 21 $x10^9/l$ at four months and 11 $x10^9/l$ at five months.

The **PLT** results estimated "graft failure" early, even with a Point of Response within the 95% limits. The **PLT** did not appear to be effected by the booster and a steady decline in

counts was seen over five months. An appearance of bimodal erythroid and granulocytic and unimodal megakaryocytic activity was evident.

4.10.2.7. Preconditioning

The preconditioning was **Cyclo/TNI**, a booster was administered six days after the "day 28" marrow biopsy. Although the patient received the same preconditioning as the **SAA** allo**BMT**, the response was different. The appearance of stromal damage resulting in poor peripheral blood counts was evident.

4.10.2.8. Mononuclear Count (MNC) and Colony forming Unit (CFU) Count

The patient's **MNC** was 7.84 $x10^8$/kg prior to the allo**BMT**, this was well above the **BMT** mean of 0.97 $x10^8$/kg. The patient's booster **MNC** was 1.09 $x10^8$/kg. The patient's **CFU-GM** count was 16.75 $x10^4$/kg prior to the allo**BMT**; this was well below the **BMT** mean of 14.19 $x10^4$/l. The booster **MNC** was far higher at 40.20 $x10^4$/kg. When compared to our findings, possible quantity of cells played a bigger role rather than quality. Because even with higher **MNC** and **CFU-GM** counts no acceptable early responses were seen in any of the blood parameters, the patient ultimately sustained "graft failure".

4.10.2.9. The Response Range

- **Reticulocyte Maturation Index (RMI)**

Only following the booster, did the **RMI** values exceed the Response Range, but only on a few occasions. **RMI** Values were excessively low prior to the booster with counts below **2SD** on numerous occasions. Although our research indicated that **RMI** values below **2SD** were of little prediction value, the patient did show values below the mean prior to the booster. The patient's **RMI** values, as an entirety did indicate poor recovery.

- **Absolute Neutrophil Count (ANC)**

The **ANC** ran below the mean for the first thirty days, and only after the booster did the counts increase to acceptable limits. However, they never increased above the mean or **2SD** at any point. Our research suggested that lower counts were of little predictory value. In this case, because the **ANC** were continuously low poor, recovery was indicated.

- **Platelet Count (PLT)**

With the exception of three days where the **PLT** increased above 100 x10^9/l all the counts remained below the mean. Even after the booster, the patient's **PLT** remained well below the mean and **2SD**. Even though our research detected no prediction value in low counts, the evidence of continual low **PLT** could be taken as an indicator for poor recovery.

4.10.2.10. "Day 14" Marrow Biopsy

The **RMI**, **ANC** and the first "day 14" biopsy revealed no engraftment. The first "day 28" marrow biopsy along with the peripheral blood counts revealed minimal granulocytic and erythroid recovery. The **PLT** however, indicated engraftment at both fourteen and twenty eight days. The second peripheral blood counts and "day 14" marrow biopsy revealed a hypocellular marrow with minimal evidence of erythrocytic and granulocytic and no megakaryocytic recovery. Granulocytic and erythrocytic bimodal response was evident along with megakaryocytic unimodal response.

4.10.3. An Acute Myeloid Leukaemia autologous Peripheral Blood Stem Cell Transplant (AML autoPBSCT)

4.10.3.1. Reticulocyte Maturation Index (RMI) Response Time

The **RMI** Response Time was essentially non existent, with a value of 0.27 IU at twenty seven days. The count decreased within a day only to fluctuate above and below 0.20 IU continuously until the day she died. The **RMI** value was 0.11 IU at forty days, 0.09 IU at three months, 0.13 IU at four months, 0.16 IU at five months and 0.08 IU at six months.

4.10.3.2. Reticulocyte Maturation Index (RMI) Point of Response

The patient never achieved an acceptable Point of Response, nor did she manage to maintain a 0.20 IU **RMI** value for any length of time. According to our research these results indicated a true "graft failure". Normal **RMI** values were occasionally observed, although there was no evidence of recovery. **RMI** values were until erythroid aplasia was reached.

4.10.3.3. Absolute Neutrophil Count (ANC) Response Time
An **ANC** Response Time of 0.50 $x10^9$/l was never achieved at any point following the transplant. The patient produced significantly lower **ANC** than the mean until she died. An **ANC** of 0.10 $x10^9$/l was seen at forty days, 0.12 $x10^9$/l at three months, 0.02 $x10^9$/l at four months, 0.02 $x10^9$/l at five months and 0.01 $x10^9$/l at six months.

4.10.3.4. Absolute Neutrophil Count (ANC) Point of Response
The patient had no Point of Response. According to our criteria she was considered a true "graft failure".

4.10.3.5. Platelet Count (PLT) Response Time
A **PLT** Response Time of 74 $x10^9$/l was seen within six days. The patient unfortunately failed to maintain a **PLT** count above 50 $x10^9$/l for more than a day at a time. Most of the counts fell below 10 $x10^9$/l within a day or two of a responsive count. A **PLT** of 13 $x10^9$/l was seen within forty days, the count was less than 10 $x10^9$/l at three, four, five and six months.

4.10.3.6. Platelet Count (PLT) Point of Response
The patient had no **PLT** Point of Response. According to our criteria she was considered a true "graft failure".

4.10.3.7. Preconditioning
Being an **AML** auto**PBSCT** the patient's preconditioning was **Bu/Cy.** Of the study group, seventy five percent of the auto**PBSCT** preconditioned with **Bu/Cy** achieved "12 month post transplant trilineage response". This alone was a strong indicator that "graft failure" could be directly linked to the preconditioning.

4.10.3.8. Mononuclear Count (MNC) and Colony Forming Unit (CFU) Count
The auto**PBSCT** group mean **MNC** was 6.01 $x10^8$/kg. The patient's **MNC** count was 5.56 $x10^8$/kg. A **MCN** of 0.45 $x10^8$/kg below the mean was possibly of little significance, with no bearing on the transplant response, because of the high medium term response that was found in most of the auto**PBSCT** patients. On the other hand the **CFU-GM** might have been a direct influence on the "graft failure", possibly because

there was no laboratory growth, indicting no viable cells to start with, hence the complete lack in response in all three parameters.

4.10.3.9. The Response Range

- **Reticulocyte Maturation Index (RMI)**

The patient failed to achieve a mean **RMI** within the first forty days. She did however, maintain a value within **2SD**. Our research revealed that lower than **2SD RMI** values were of no value when predicting post transplant recovery. The patient experienced the same **RMI** pattern as the second **SAA** allo**BMT.** Neither produced **RMI** values higher or lower than **2SD**, and the counts retained below average for the first forty days. This allowed for the possibility of poor recovery.

- **Absolute Neutrophil Count (ANC)**

The patient never achieved a mean **ANC** within the first forty days. Although her counts remained within **2SD**, this was of little consequence as the **2SD** was 0.00 $x10^9$/l. Our research revealed that **ANC** below **2SD** within the first forty days were of little consequence, however, a healthy response was important. In this case the constantly low **ANC** did allow for the possibility of poor recovery.

- **Platelet Count (PLT)**

On occasion the **PLT** rose above the mean, the remainder of the time they fell below. Although the patient's **PLT** values never decreased to **2SD** (0.00 $x10^9$/l), they regularly fell below 10 $x10^9$/l. Our research indicated that **PLT** counts below the mean within the first forty days were of little value. The patient's continual low counts, below 10 x9/l was most likely an indication of poor recovery.

4.10.3.10. "Day 14" Marrow Biopsy

The "day 14" marrow biopsy results along with the peripheral blood counts, indicated aplasia. Although the "day 28" marrow biopsy suggested a small amount of granulocytic response, the peripheral blood counts indicated nothing. The remainder of the marrow biopsies and the peripheral blood counts revealed "graft failure".

4.10.4. A Severe Aplastic Anaemia allogenic Peripheral Blood Stem Cell Transplant (SAA alloPBSCT)

4.10.4.1. Reticulocyte Maturation Index (RMI) Response Time

The **RMI** Response Time was ten days following the first transplant. These counts fell below 0.20 IU within a day and remained below this point for fifty two days, the occasional increase was however observed. Following the booster, the **RMI** Response Time was six days.

4.10.4.2. Reticulocyte Maturation Index (RMI) Point of Response

The allo**PBSCT** group **RMI** Point of Response was sixteen days. Technically the patient responded within a 95% limit following the first transplant. However, he failed to maintain acceptable **RMI** values, resulting in an erythrocytic "graft failure". The **RMI** Point of Response following the booster was six days, thereafter counts remained above 0.20 IU. The patient, being a child, only remained at Groote Schuur for twenty five days before being returned to Red Cross Children's Hospital. Although he achieved a "12 month post transplant trilineage response", no blood was available for analysis after he was transferred.

4.10.4.3. ANC Response Time

The allo**PBSCT** group mean **ANC** Response Time was thirteen days. No **ANC** response was seen following the first transplant. The patient never achieved a count higher than 0.06 $x10^9/l$ for fifty two days. An **ANC** Response Time of eleven days following the booster was observed, and a count of 5.55 $x10^9/l$ at day twenty five.

4.10.4.4. Absolute Neutrophil Count (ANC) Point of Response

The allo**PBSCT** group **ANC** Point of Response was twenty nine days. There was no Point of Response following the first transplant. After the booster, a response was seen at eleven days, which fell within the 95% limit.

4.10.4.5. Platelet Count (PLT) Response Time

No acceptable **PLT** Response Time was seen following the first transplant. At times the **PLT** rose above 50 $x10^9/l$ only to fall again, resulting in an erratic picture with no stable increase. The **PLT** response did appear to be healthier than either the **RMI** or **ANC**

response; this was possibly due to **PLT** transfusions. A **PLT** Response Time of nine days following the booster was observed, and a count of 112 $x10^9$/l at twenty five days recorded.

4.10.4.6. Platelet Count (PLT) Point of Response

The allo**PBSCT** group **PLT** mean Point of Response was thirty three days. The patient indicated no acceptable response following the first transplant. This resulted in no indication of megakaryocytic recovery, "graft failure" was assumed. A Point of Response was seen within nine days following the booster, one day prior to the **ANC** response, and only three days after the **RMI** response. The patient's **PLT** response fell within the 95% limit.

4.10.4.7. Preconditioning

Cyclo/Alg was the preconditioning of choice prior to the first transplant, followed by a booster after fifty two days. The preconditioning was the same as the **SAA** allo**BMT**'s. However, the allo**PBSCT** child was T-Cell depleted, this may have played a role in the fact that he achieved a "12 month post transplant trilineage response". Although there was an indication indication of minimal stromal damage in any of the **SAA** transplants, it could be possible that this particular patient sustained less damage than the other two.

4.10.4.8. Mononuclear Count (MNC) and Colony Forming Unit (CFU) Count

The mean **MNC** for the allo**PBSCT** group was 7.51 x108/kg. The patient produced a **MNC** count of 9.48 $x10^8$/kg for the transplant and 10.60 $x10^8$/kg for the booster. Our research found that more than fifty percent of the allo**PBSCT** with higher **MNC** produced early peripheral blood count parameters. In this patient, both the transplant and booster **MNC** were higher. The transplant failed to engraft.

The mean **CFU-GM** for the allo**PBSCT** group was 31.25x104/kg. The patient produced a **CFU-GM** of 6.60 $x10^4$/kg for the transplant and 34.80 $x10^4$/kg for the booster. Our research indicated that higher **CFU-GM** counts produce early peripheral blood responses in less than forty five percent of the patients. There was an exception in the **PLT** parameter however, with seventy seven percent, although most of the patients died. In

this case higher counts appeared to generate early responses, and resulted in a positive medium term response.

4.10.4.9. The Response Range

- ***Reticulocyte Maturation Index (RMI)***

The **RMI** counts failed to reach the mean at any point during the first fifty two days. Following the booster, the counts rose above the mean from the day of response. Although the Point of Response was the indicator for "graft failure", there was no visible **RMI** increase according to the established range, this indicated a poor erythroid recovery.

- **Absolute Neutrophil Count (ANC)**

The **ANC** were below the mean throughout the first fifty two days. Following the booster the counts rose above the mean and the **2SD** immediately after the response date. Like the **RMI**, the continuous low **ANC** following the first transplant, was an indication of poor granulocytic recovery.

- **Platelet Count (PLT)**

The **PLT** response was different to the **RMI** and **ANC** response. Although estimating "graft failure" following the fist transplant, the **PLT** was not always below the mean like the **ANC**. This indicated that erythroid and granulocytic recovery were bimodal and the megakaryocytic was unimodal. Another explanation for superior **PLT** response could be that the megakaryocytic response was relative to the **CFU-GM** count. Although the first **CFU-GM** count was below the mean, progenitor cell richness may have affected the **PLT**. Following the booster **PLT** above the mean and **2SD** were seen.

4.10.4.10. "Day 14" Marrow Biopsy

No marrow biopsies were performed at Groote Schuur Hospital.

CHAPTER 5

Discussion

5.1. Introduction

Comparative studies between various flow cytometers using Thiozole Orange (**TO**) and traditional manual methods have been performed. These have expressed excellent results, proving that flow cytometric reticulocyte analysis is accurate, time saving and cost effective (Carter *et al*,1989;Lee *et al*,1986). By 1990 **TO** was commercially available as Retic-**COUNT**, a product by Becton Dickinson. Although automated reticulocyte counts were higher than manual counts, they had the advantage of less fluctuation, and a prolonged storage period of seventy two hours prior to testing (Cavill *et al*,1996;Chin-yee *et al*,1991;Davis *et al*,1990). The best correlation was seen in reticulocyte counts below one percent, which added to the attraction of monitoring recovery of the bone marrow following myeloablation (Carter *et al*,1989;Ferguson *et al*,1990).

Various reticulocyte parameters have been used to evaluate erythrocyte response in Bone Marrow Transplant (**BMT**) and Peripheral Blood Stem Cell Transplant (**PBSCT**) patients. Parameters such as Reticulocyte Percentage, Mean Channel Rate (**MCR**) or Mean Fluorescent Index (**MFI**), High Fluorescing Reticulocytes (**HFR**), the sum of the Medium and the High Fluorescent Reticulocytes (**MFR-HFR**) and the Reticulocyte Maturation Index (**RMI**) were established.

Davis *et al* (1989) introduced the **MFI** as a measurement of reticulocyte response in patients undergoing autologous Bone Marrow Transplant (auto**BMT**). They found that patients with normal reticulocyte percentages produced a wide range of **MFI** results, this was due to the fact that the **MFI** was based purely on the Mean Channel Rate (**MCR**), and shifts involving the mean were overly sensitive. In a second study by Davis *et al* (1989) it was concluded that erythrocyte response using **MFI** was detected at least three days earlier than the **ANC** for granulocyte response. In a further publication Davis *et al*

(1990) postulated that the **MFI** could identify three patterns of marrow engraftment, early, delayed and failed. In 1991 Chin-yee *et al* also studied the **MFI.** Only four of the ten auto**BMT** patients revealed an earlier response in **MFI** prior to **ANC** response. Large amounts of **MFI** fluctuations were found between patients. This fluctuation was believed to be due to low reticulocyte counts. **MFI** representing the average fluorescence of very few events increased the risk of sampling error. There was a lack of standardisation to ensure stability within the technique. Because of these published findings we decided that the **MFI** was not the method of choice for the Coulter **EPICS Profile II**.

In 1992 Davis *et al* evaluated **HFR** as an indicator for allogenic and autologous **BMT** response. They found a significant **HFR** increase in allogenic Bone Marrow Transplant (allo**BMT**) on day twelve and auto**BMT** on day fourteen. In 1994 Greinix *et al* also used the **HFR** as a prediction tool in **BMT**. Their patients had a **HFR** response at fourteen days for allogenic and fifteen days for autologous transplants. d'Onofrio *et al* (1996) studied a group of **BMT**, which indicated a thirteen day **HFR** median response. Testa *et al* in their 1997 publication found a peak response at day eight in **PBSCT** patients, and twenty four days in Bone Marrow Stem Cell Transplant (**BMSCT**) patients. In 2000 George *et al* found an eight day **HFR** mean and median response following autologous Peripheral Blood Stem cell Transplant (auto**PBSCT**). Although the **HFR** was the most commonly used parameter for detecting reticulocyte response post transplant, the method was not favourable for our purpose. The Coulter **EPICS Profile II** flow cytometer only reports **HFR** as a percentage. In the case of an exceptionally low reticulocyte count, the **HFR** would be reported as zero. Unfortunately when monitoring low reticulocyte counts and their recovery, a zero percentage is not practical.

In 1994 Batjier *et al* introduced the **MFR+HFR** as a reticulocyte response indicator. Their study revealed a mean response at thirteen days post auto**BMT**. However, Remacha, one of the researchers in allo**PBSCT**, found faster recoveries in **PBSCT**. For the purpose of our study, using the percentage recovery on the Coulter **EPICS Profile II** flow cytometer was not practical, again because both parameters are reported in percentage.

In 1992 Davis *et al* introduced the **RMI** by evaluating eleven different instruments. They established a normal range of 0.20 to 0.50 IU. Their 1993 publication described in detail how they achieved the **RMI** values. In our study we have applied this method. In 1996 Dahal *et al* used the Davis **RMI** value to measure erythrocyte response in allo**BMT** and auto**BMT,** they obtained a median response of seventeen days. To date the **RMI,** as an indicator for erythroid response was the least used of all the methods. For our purpose the **RMI** was the most practical means of measuring the earliest reticulocytes on the Coulter **EPICS Profile II** flow cytometer.

5.2. Reticulocyte Maturation Index (RMI) as an Indicator for Reticulocyte Response

The **RMI** we believe is the most accurate means of measuring an early reticulocyte response on the **COULTER Profile II** flow cyto neter. When the flow cytometer records the number of events (usually fifty thousand), it calculates the percentage high fluorescing reticulocytes in the total number of events. These events, not the percentage, were used to calculate the **RMI**. For the purpose of a very low immature reticulocyte count, such as post bone marrow ablation, a minimal number of immature reticulocytes in relation to the events would be reported. Small numbers of immature reticulocytes yield results. An **RMI** of zero is very rarely reported, unlike the **HFR** (Testa *et al*,1997). As soon as immature reticulocytes enter the peripheral blood, the **RMI** increases. In the case of a **COULTER Profile II** this would be prior to the **HFR%**.

The **MFI** or **MCR** is based on the mean channel reading of the reticulocyte distribution curve, which shifts to the left or right depending on the **RNA** content. The mean may not move by any significant amount when only small numbers of immature reticulocytes enter the peripheral blood, resulting in less than one hundred per cent erythroid response seen prior to granulocytic response (Davis *et al*,1989). The **RMI** response post transplant is Gaussian (Figure 6 Page 40), proving that the **RMI** is the most stable parameter to monitor post transplantation. All techniques used for monitoring early reticulocyte response post bone marrow transplant are subject to standardisation (Chin-Yee *et al*,1991). Because numerous instruments are marketed with a variety of techniques that

may be applied, each laboratory should be responsible for establishing their own **RMI** values and instrument standardisation (Davis *et al*,1990).

5.3. The Mean Response Time

5.3.1. Reticulocyte Maturation Index (RMI)

All literature examined indicated a reticulocyte parameter response ranging from eight to seventeen days. They were group specific. Most of the publications concentrated on auto**BMT** with responses of eighteen days (Davis *et al*,1989), fourteen days (Davis *et al*,1992), twelve days (Greinix *et al*,1994), fourteen days (d'Onofrio *et al*,1996), twenty four days (Testa *et al*,1997), thirteen days (Batjer *et al*,1994) and seventeen days (Dahal *et al*,1966). Our study contained no auto**BMT**. Findings published on allo**BMT**, reticulocyte parameter responses were seen at twelve days (Davis *et al*,1992), eleven days (d'Onofrio *et al*,1996), thirteen days (Bajer *et al*,199[illegible]), seventeen days (Dahal *et al*,1996) and seven days (Greinix *et al*,1994).

Of the eleven different groups analysed five produced an eight day **RMI** mean Response Time. All the transplants responded within nine days (Figure 5 Page 39), with counts varying from 0.33 IU to 0.45 IU at forty days from 0.16 IU to 0.26 IU at six months. Once the patients in our study were separated, the **BMT** achieved an earlier **RMI** Response Time at eight days compared to the **PBSCT** at nine days (Figure 5 Page 39). The two produced similar counts at forty days, 0.45 IU and 0.38 IU respectively. Within six months the **PBSCT** resulted in a **RMI** decrease almost three times that of the **BMT.** The **BMT** showed a normal **RMI** value at six months of 0.33 IU, and the **PBSCT** a lower than normal value of 0.19 IU (Figure 10 Page 46). **BMT** erythroid recovery was more stable during the first six months post transplant. The stability may have been due to the quality of infused stem cells.

The allogenic and autologous transplants revealed the same mean Response Time at nine days, with **RMI** values at forty days of 0.37 IU and 0.41 IU respectively. The autologous transplants produced a percentage decrease double that of the allogenic transplants within six months, one hundred and fifty six compared to forty two percent. Seventy seven percent of the autologous transplants and only thirty four percent of the allogenic

transplants achieved a "12 month post transplant trilineage response". We found that attaining a mean Response Time to detect erythrocytic recovery was just as important as monitoring an erythrocyte response for the first forty days.

Published data on auto**PBSCT** revealed reticulocyte parameter responses, at eight days (George *et al*,2000;Testa *et al*,1997) and nine days (Torres *et al*,2001). These responses were in agreement with our research, at a nine day mean Response Time in auto**PBSCT**. There was one publication on allogenic Peripheral Blood Stem Cell Transplant (allo**PBSCT**) which reported **RMI** parameter response within thirteen days (Torres *et al*,2001). This did not compare well with our eight day mean Response Time. The allo**PBSCT** in our study revealed an **RMI** mean Response Time at eight days and a 0.33 IU count at forty days. The percentage decrease over six months was only forty two percent, with the highest **RMI** value in all the groups of 0.26 IU. The auto**PBSCT** revealed a day nine **RMI** mean Response Time, 0.40 IU count at forty days and a one hundred and fifty percent decrease over six months. Although the allo**PBSCT** revealed a more stable six month recovery, the auto**PBSCT** surpassed them, producing a seventy seven percent "12 month post transplant trilineage response" achievement. It was evident that although an early mean Response Time indicated erythroid recovery, patients needed to achieve a healthy **RMI** count within the first forty days to correct for the anaemia following transplantation and to sustain life during the erythroid production decrease during the medium term.

The Acute Myeloid Leukaemia allogenic Peripheral Blood Stem Cell transplant (**AML** allo**PBSCT**) group produced the earliest **RMI** Response Time of all the groups at seven days, but the lowest count at forty days (0.33 IU) and six months (0.16 IU). Only twenty nine percent of them achieved a "12 month post transplant trilineage response". The Chronic Myeloid Leukaemia allogenic Peripheral Blood Stem Cell Transplant (**CML** allo**PBSCT**) group produced the slowest **RMI** Response Time of all the groups, at eleven days, with one of the highest **RMI** values at forty days (0.45 IU) and six months (0.28 IU). They only achieved a twenty two percent "12 month post transplant trilineage response". The Acute Myeloid Leukaemia autologous Peripheral Blood Stem cell

Transplant (**AML** auto**PBSCT**) group, with a slow **RMI** Response Time at ten days, a good forty day **RMI** value (0.41 UI) was seen followed by a rapid decrease within six months (0.16 IU). This group still achieved a sixty seven percent "12month post transplant trilineage response". When the smaller more specific groups were analysed, it became apparent that the **CML** transplants, which are known slow **ANC** responders, were proven to be slow erythrocyte responders as well (eleven days). Although the allogenic **AML** transplants were superior responders, the autologous transplants definitely produced a higher medium term success rate (seventy seven percent).

The "12 month post transplant trilineage response" group, with an average mean Response Time of eight days, produced one of the highest **RMI** values within forty days (0.41 IU) and one of the highest percentage decreases over six months at one hundred and forty one percent (0.17 IU).

All eleven transplant groups produced steady **RMI** increases, ranging between 0.33 and 0.41 IU within the first forty days; a mean decrease between 0.16 and 0.33 IU was seen within six months. We need to take into account medium to long term expectancy, erythrocytes produce macrocytosis and hypochromia with subnormal bone marrow cellularity, even with no evidence of recurrent disease. This possibly is as a result of the donor cells repopulating the recipient's marrow and an expansion of haemopoietic tissue to atypical medullary sites (Arnold *et al*,1989). Another consideration in allogenic transplants is that erythroid generation is compromised in **ABO** incompatibility (Dahal *et al*,1996).

The **RMI** Response Time proved to be an excellent tool for establishing an early erythroid recovery post transplant. Of the eleven groups analysed, the **RMI** mean Response Time was the first parameter to recover. It was the most stable parameter of the three during the first forty days (Graph 1 Page 46). A mean **RMI** Response Time of eight days for the majority of the groups was evident. This was earlier than most published results (Batjer *et al*,1994; Dahal *et al*,1996;Davis *et al*,1992;d'Onofrio,1996;Greinix *et al*,1994). The forty five successful engraftments

generated **RMI** values of 0.20 IU within seventeen days. The four "graft failures" either failed to achieve a response within seventeen days or maintain a count above 0.20 IU for any length of time within the first forty days. This discovery leads us to believe that if a patient does not achieve an **RMI** response within seventeen days post transplant, primary "graft failure" is highly likely.

Although the mean **RMI** Response Time indicted no medium term prediction value, it was important for the marrow to generate an erythrocyte recovery well within the normal range for the first forty days. It was of little consequence that once the anaemia was corrected and the **RMI** values decreased, successful engraftment had been achieved and progenitor cells were well placed.

5.3.2. Absolute Neutrophil Count (ANC)

The mean Response Time for the **ANC** in our study ranged between thirteen and twenty days depending on the type of transplant. Our results were in agreement with other publications. Davis *et al* (1989) detected **BMT ANC** responses between ten to thirty four days; Greinix *et al* (1994) discovered a median **BMT** response of nineteen days, Batjer *et al* (1994) fifteen days, and d'Onofrio *et al* (1996) a **BMT** median of eighteen days.

The transplants as a group produced an **ANC** mean Response Time of sixteen days with counts between 0.12 $x10^9$/l and 2.65 $x10^9$/l within the first forty days, and 2.52 $x10^9$/l to 3.03 $x10^9$/l within six months. This resulted in an average seventy percent increase. Fluctuations within the counts were seen, however, most remained above the mean. The **ANC** counts varied from the **RMI** response by increasing over six months.

The **BMT** produced one of the slowest mean **ANC** Response Times at seventeen days, but the highest **ANC** at forty days (2.65 $x10^9$/l). Although the **PBSCT** produced a slightly faster mean Response Time at fifteen days, the count was lower at forty days (1.13 $x10^9$/l). The percentage recovery was one hundred and fifty percent higher in the **PBSCT** than in the **BMT** with counts at 2.82 $x10^9$/l and 2.64 $x10^9$/l respectively within six months. The higher **BMT ANC** counts were possibly due to a superior number and richness of stem cells harvested. Another factor could be a result of different T-Cell

depletion methods used between various transplants, resulting in slower **PBSCT** medium term recovery (Hale *et al*,1983).

The allogenic transplants generated an eight day mean Response Time compared to the autologous transplants at nine days. Although the autologous transplants produced a lower **ANC** at forty days (1.12 $x10^9$/l) compared to the allogenic transplants (1.63 $x10^9$/l); the autologous transplants were far less stable during the first forty days (Graph 10 Page 61). The percentage increase over six months of the autologous transplants was three times higher than the allogenic transplants (one hundred and seventy one percent compared to sixty two percent), with seventy seven percent achieving a "12 month post transplant trilineage response". Although the allogenic transplants generated earlier responses and steadier **ANC** over a six month period, the autologous transplants produced a healthier medium term prognosis. This was possibly due to the affiliation that autologous cells have for their own following transplant, which we termed a "pattern of recognition".

The allo**PBSCT** and the auto**PBSCT** generated a mean **ANC** Response Time of eight and nine days respectively, with the same counts at forty days (1.13 $x10^9$/l and 1.12 $x10^9$/l). The auto**PBSCT** produced the highest percentage increase over six months (one hundred and seventy one percent), resulting in a count of 3.03 $x10^9$/l. This was possibly due to the "pattern of recognition" discovered in the autologous transplants.

Although the **AML** allo**PBSCT** produced the earliest **ANC** mean Response Time at fourteen days, the recovery from forty days to six months was unstable (1.13 $x10^9$/l to 2.78 $x10^9$/l), with a seventy five percent death rate. The **CML** allo**PBSCT** produced the slowest mean Response Time at twenty days, the lowest **ANC** at forty days (0.12 $x10^9$/l) and the highest increase of all at two thousand percent within six months (2.52 $x10^9$/l). The significance of the rapid increase may be due to the fact that five of the seven patients died three of them relapsing. **CML** produce higher numbers of relapses because T-Cell depletion inhibits the graft versus leukaemia effect. The **AML** auto**PBSCT** group produced the third highest mean Response Time at ten days, with an average **ANC** at

forty days of 0.12 $x10^9/l$ and 2.91 $x10^9/l$ within six months. Sixty six percent of the patients achieved a "12 month post transplant trilineage response". This was possibly due to the "pattern of recognition".

The "12 month post transplant trilineage response" group produced a mean Response Time of fourteen days, one of the highest **ANC** at forty days (1.94 $x10^9/l$) and only a fifty percent increase over six months (2.92 $x10^9/l$). This was the only group that produced an improved **ANC** each month. This discovery possibly is of importance when monitoring post transplant patients; a healthy increase of **ANC** for the first six months would most likely verify transplant success.

The **ANC** mean Response Time was found to be an excellent tool for assessing granulocytic recovery. It was important for the **ANC** to increase within the first forty days, to allow for the stem cell engraftment and to ensure granulocytic recovery. The **ANC** was of very little use in predicting medium term recovery. However, some cases, particularly those of the "12 month post transplant trilineage response" group, lead us to believe that a healthy response during the six month post transplant period was important in achieving medium term success.

5.3.3. Platelet Count (PLT)

The **PLT** mean Response Time varied between fourteen and twenty one days. Counts of 40 $x10^9/l$ to 76 $x10^9/l$ were documented at forty days and 115 $x10^9/l$ to 164 $x10^9/l$ within six months.

Analysis of all the transplant groups revealed a **PLT** mean Response Time of sixteen days, with a count of 60 $x10^9/l$ at forty days and 141 $x10^9/l$ at six months. **PLT** fluctuations were common, and they rose and fell above and below 50 $x10^9/l$ throughout the six month period. This was possibly due to frequent platelet transfusions. The allogenic transplants produced a mean Response Time of sixteen days, whereas the autologous transplants produced a mean Response Time of seventeen days. The **PLT** parameter in the allogenic transplant group was healthier, with a forty day count of 58 $x10^9/l$ increasing to 150 $x10^9/l$ at six months. The autologous transplants produced a

count of 40 $x10^9/l$ at forty days and 115 $x10^9/l$ at six months. Although the allogenic transplant group generated the higher count, the autologous transplant percentage increase was greater (one hundred and eighty eight compared to one hundred and fifty nine percent).

The **BMT** mean Response Time was seventeen days, with a count of 78 $x10^9/l$ at forty days and 137 $x10^9/l$ at six months. The **PBSCT** mean Response Time was sixteen days, with a count of 44 $x10^9/l$ at forty days and 137 $x10^9/l$ at six months. The **PBSCT** percentage increase over six months was more than double that of the **BMT**, with the same results at six months (**PBSCT** at two hundred and eleven percent compared to the **BMT** at seventy six percent). Although the **BMT** produced more stable transplants medium term, the **PBSCT** produced far superior early responses.

The allo**PBSCT** produced one of the faster **PLT** mean Response Times at twelve days compared to the auto**PBSCT** at seventeen days. Although both transplants produced similar **PLT** counts at forty days (48 $x10^9/l$ for the allo**PBSCT** and 40 $x10^9/l$ for the auto**PBSCT**), the allo**PBSCT** produced the healthiest percentage recovery of all the groups at two hundred and twenty seven percent (157 $x10^9/l$). As a result the allo**PBSCT** produced a higher count at forty days and a highest percentage increase at six months. The allo**PBSCT** was the superior medium term responder with a higher six month count (157 $x10^9/l$). Unfortunately, only twenty three percent achieved a "12 month post transplant trilineage response".

The **AML** allo**PBSCT** produced the earliest **PLT** mean Response Time at eleven days, with a percentage increase of one hundred and seventy seven percent within six months (48 $x10^9/l$ to 163 $x10^9/l$). The **CML** allo**PBSCT** produced the slowest **PLT** mean Response Time of twenty one days, with a mean percentage increase of one hundred and twenty within six months (64 $x10^9/l$ to 161 $x10^9/l$). The **AML** auto**PBSCT** produced the second slowest **PLT** mean Response Time at nineteen days, with a mean percentage increase of one hundred and seventy one percent within six months (36 $x10^9/l$ to 163 $x10^9/l$). The **PLT** response was similar to the **ANC** response in that the **AML** allogenic

transplants were superior early responders and the **CML** allogenic transplants were the slowest responders. The autologous transplants were the slowest responders with the best medium term outcome.

The "12 month post transplant trilineage response" group generated the fastest mean Response Time at twelve days along with the allo**PBSCT**. They produced the highest mean count at forty days (76 $x10^9$/l) and the highest mean count at six months (164 $x10^9$/l). The percentage increase was one hundred and sixteen percent.

The **PLT** measurements were the least stable of the three parameters. The achievement of an adequate count estimated engraftment but was unreliable due to fluctuations. It was however, important for counts to increase steadily over six months to reach acceptable limits. The "12 month post transplant trilineage response" group indicated that an early **PLT** Response Time, along with a steady **PLT** recovery within forty days continuing for six months, was an indication of a positive medium term success. However, we need to be aware that the auto**PBSCT** with the lowest count at forty days (40 $x10^9$/l) and six months (115 $x10^9$/l), generated a seventy seven percent "12 month post transplant trilineage response" achievement.

5.4. Point of Response

5.4.1. Reticulocyte Maturation Index (RMI)

All the patients responded within seventeen days post graft infusion. The Point of Response for six of the eleven groups was sixteen days, with a range of ten to sixteen days. The **RMI** was the fist parameter to respond in all the patients.

The Point of Response for the transplants as a group was sixteen days. Two patients responded on day seventeen, however they were not considered "delayed responses". The allogenic transplants' Point of Response was fifteen days and the autologous transplants sixteen days. When compared to the **PBSCT** Point of Response of sixteen days, the **BMT** was fifteen days. Once the **PBSCT** was divided, the allo**PBSCT** Point of Response was sixteen days and auto**PBSCT** seventeen days. The **AML** allo**PBSCT** Point of Response was seventeen days, the **CML** allo**PBSCT** response sixteen days, the

AML auto**PBSCT** response fourteen days and the "12 month post transplant trilineage response" group was fourteen days. The **RMI** Point of Response encompassed a very tight group of numbers with very little leeway for indicating "graft failure". The **RMI** recovery needed to be recorded within seventeen days to indicate engraftment; otherwise primary "graft failure" was to be considered. According to the **AML** allo**PBSCT** and the "12 month post transplant trilineage response" group, a response of fourteen days indicated recovery along with the possibility of a positive medium term prognosis.

Of the "graft failures", only one patient achieved **RMI** values of 0.20 IU within the first seventeen days. He however, never maintained the count for longer than a day at a time. All four of the patients indicated a "graft failure" due to a lack of **RMI** response within the first seventeen days. Following the second transplant and boosters, the only patient to indicate **RMI** recovery, was the Severe Aplastic Anaemia allogenic Peripheral Blood Stem Cell Transplant (**SAA** allo**PBSCT**), who achieved a "12 month post transplant trilineage response".

Transplants should achieve a **RMI** of 0.20 IU or more within seventeen days post infusion, to indicate a successful engraftment. The **RMI** would be expected to rise to 0.39 IU within forty days, ultimately decreasing, reaching a mean of approximately 0.19 IU within six months. Our research indicated that any patient with an **RMI** response later than seventeen days faces potential primary "graft failure". All of the patients within the study who engrafted had an **RMI** response within seventeen days.

5.4.2. Absolute Neutrophil Count (ANC)

The transplants as a group produced an **ANC** Point of Response of twenty nine days. Two patients fell outside the limit, and responded on day thirty and thirty two. They were not considered "delayed responses". One patient who was not used in the calculation responded on day fifty seven, although she was considered a "delayed response", she achieved a "12 month post transplant trilineage response".

The allogenic transplant group **ANC** Point of Response was thirty days and the autologous transplant group twenty one days. Although the allogenic transplants

produced an earlier mean Response Time (sixteen days compared to seventeen days) the autologous transplants produced the earliest Point of Response (twenty one days). In addition, they produced a healthier **ANC** recovery within six months. This was possibly due to the "pattern of recognition".

Our findings were in keeping with published data. The **BMT** sustain a prolonged aplastic phase compared to **PBSCT** (d'Onofrio *et al*,1996). The **PBSCT ANC** Point of Response was twenty one days compared to the **BMT** of thirty two days. The **PBSCT ANC** mean Response Time and Point of Response were both faster than the **BMT** response. As a result, the **PBSCT's** were superior responders.

The allo**PBSCT ANC** Point of Response was twenty nine days while the auto**PBSCT** was twenty one days. Although the allo**PBSCT** and auto**PBSCT** mean **ANC** Response Time was similar (thirteen and fifteen days), the autologous transplants revealed a definite "pattern of recognition", which not only produce positive medium term successes but healthier counts within six months. The **AML** allo**PBSCT ANC** Point of Response was thirty days and the **CML** allo**PBSCT** twenty eight days. The **AML** auto**PBSCT** Point of Response was twenty one days, again depicting a "pattern of recognition" (d'Onofrio *et al*,1996;Testa *et al*,1997). Because the "12 month post transplant trilineage response" group contained both allogenic and autologous transplants, the **ANC** Point of Response was twenty six days.

According to the literature, failure to obtain an **ANC** count of 0.50 $x10^9/l$ within twenty days could be considered primary "graft failure". A number of publications consider this too low (Davis *et al*,1992;Greinix *et al*,1994). A **NHL** allo**BMT** who responded on day thirty two achieved a "12 month post transplant trilineage response". A **CML** allo**PBSCT** responded on day fifty seven. She would be considered a "graft failure", however the patient achieved a "12 month post transplant trilineage response". In contrast, an **AML** allo**PBSCT** with a day thirty response, a **CML** allo**PBSCT**, also with a day thirty response and an **AML** auto**PBSCT** with a day twenty one response, all died. Of the four "graft failures", the first **SAA** allo**BMT** achieved a twenty five day response.

Following the second transplant, the patient died. The **SAA** allo**PBSCT** only achieved a response following the booster. The other two patients never achieved or maintained an **ANC** of 0.50 $x10^9/l$ for any length of time, therefore, indicating "graft failure". Thus, there is a definite need for revaluation of failure criteria. A Point of Response should be calculated for each group separately.

Although the **ANC** Response Time was found to be an excellent tool for indicating granulocytic engraftment, there was little prognostic value attached for medium term recovery. The **ANC** Point of Response appeared to be of better prognostic value than the **ANC** Response Time, particularly when using the patient's recovery to project a possible outcome. The **ANC** Point of Response created a tool to establish a framework for confidence limits and allow for speculation of "graft failure". Both methods should be used simultaneously when monitoring transplant patients.

5.4.3. Platelet Count (PLT)

The patients as a group revealed a **PLT** Point of Response within thirty three days. One patient produced a "slightly delayed response" at thirty six days. The allogenic transplant group **PLT** Point of Response was thirty three days and the autologous transplants twenty four days. Both the **PBSCT** and the **BMT PLT** Point of Response were thirty three days. Although **PLT** in the autologous transplants appeared to entertain the "pattern of recognition", counts were not stable for at least a month post transplant due to platelet transfusions. This instability was in finding with other publications (d'Onofrio *et al*,1996).

The allo**PBSCT PLT** Point of Response was thirty three days, and the auto**PBSCT** twenty four days. The autologous "pattern of recognition" was evident, within the megakaryocytic line. The **AML** allo**PBSCT PLT** Point of Response was thirty two days. The **CML** allo**PBSCT** was thirty six days, which was the longest of all the groups analysed. The **AML** auto**PBSCT PLT** Point of Response was twenty one days, the fastest of all the groups. Not only was the "pattern of recognition" indicated in the autologous transplants, but was even more evident in the **AML** autologous transplants.

The "12 month post transplant trilineage response" produced a **PLT** Point of Response within thirty three days.

More patients fell outside the 95% Point of Response, in the **PLT** parameter than the **RMI** or **ANC**. A **CML** allo**PBSCT** produced a slightly "delayed response" at thirty six days, an **AML** allo**PBSCT** "delayed response" at day thirty three and a **CML** allo**PBSCT** responded on day thirty six. All these patients achieved a "12 month post transplant trilineage response". Two "delayed responses", a **CML** allo**BMT** who responded on day seventy three and a **CML** allo**BMT** patient who responded on day forty six, both died. To summarise, of the five patients that fell outside the 95% Point of Response, two died, both with **PLT** response times of greater than thirty days. However, the two with greater than thirty six day responses, achieved a "12 month post transplant trilineage response".

Both the Severe Aplastic Anaemia allogenic Bone Marrow Transplant (**SAA** allo**BMT**) "graft failure", revealed **PLT** responses within acceptable time limits, however, counts were not maintained for more than three days at a time. Following a second transplant and booster, they never reached 50 $x10^9/l$. The **AML** auto**PBSCT** only produced occasional counts above 50 $x10^9/l$. The **SAA** allo**PBSCT** indicated no **PLT** response following the first transplant, but after the booster a response was seen within nine days. He was the only patient to achieve a "12 month post transplant trilineage response".

With a wide divergence of Point of Responses seen within the **PLT**, it was obvious that the parameter was not a reliable indication of engraftment. Although the **PLT** Point of Response did not appear to reveal as much information as the **RMI** or **ANC**, the tendency to involve the "pattern of recognition" within the autologous transplants was evident. In most cases, for the **PLT** to be part of the trilineage recovery, there was a need to respond within thirty six days.

5.5. Preconditioning

Sixty three percent of the patients were preconditioned with **Cyclo/TBI/TNI**; forty three percent were **PBSCT** and twenty percent **BMT**. When analysing all the transplant groups together approximately, seventy percent of the patients generated early responses in all three parameters, only forty percent of them achieved a "12 month post transplant trilineage response". Once the groups were subdivided, the allogenic transplants produced early responses in approximately sixty five percent of the patients, with only forty percent achieving a "12 month post transplant trilineage response". About sixty percent of the autologous transplants produced early responses; one hundred percent achieved a "12month post transplant trilineage response". On separation, seventy percent of the **PBSCT** produced early responses compared to sixty percent of the **BMT**. An improved "12 month post transplant trilineage response" was seen in the **PBSCT** with almost fifty percent success compared to the thirty percent in the **BMT**.

When the **PBSCT** were subdivided, a more specific pattern emerged. The allo**PBSCT** revealed seventy percent early **RMI** responses, and sixty percent in the **ANC** and **PLT** parameters. Less than thirty percent of them achieved a "12 month post transplant trilineage response". The auto**PBSCT** however, only generated a fifty percent early response in all three parameters; one hundred percent of them achieved a "12 month post transplant trilineage response". Once the smaller specific groups were established, all three, the **AML** allo**PBSCT**, the **CML** allo**PBSCT** and the **AML** auto**PBSCT** revealed about sixty percent early responses in all three parameters. Both the **AML** allo**PBSCT** and the **CML** allo**PBSCT** achieved less than fifty percent "12 month post transplant trilineage response" compared to the **AML** auto**PBSCT** at one hundred percent. More specific early responses were seen in the "12 month post transplant trilineage response" group, with a hundred percent success rate.

To summarise **Cyclo/TBI/TNI** was the preconditioning of choice in most patients, with early responses seen in all three parameters in approximately sixty percent of them. The **RMI** produced a high percentage of about seventy percent early responses particularly in auto**PBSCT**. **ANC** and **PLT** responses were particularly good in the allo**PBSCT** at more

than sixty percent. These numbers indicate that **Cyclo/TBI/TNI** preconditioning does not appear to adversely damage the stroma. Only forty percent of the patients with early responses achieved a "12 month post transplant trilineage response". There were however some patients, particularly in the auto**PBSCT** group, with one hundred percent, and the **BMT**, with only thirty percent "12 month post transplant trilineage responses". These positive and poor negative results for medium term success were possibly not related to stromal damage, but more likely to do with the quality and richness of **MNC** and **CFU-GM** counts.

Bu/Cy as a preconditioning regime was used on eighteen percent of the patients. All of them were allogeneic transplants. Only twenty two percent of the patients produced an early **RMI** response compared to fifty percent early **ANC** response and forty percent early **PLT** response. Sixty percent of them however, achieved a "12 month post transplant trilineage response". Although **Bu/Cy** appeared to produce a greater effect on the erythroid response, than the other two, no consequences were indicated in the medium term.

Bu/Mel/Thiotep was used on eight percent of the patients, with only twenty percent of them achieving a "12 month post transplant trilineage response". These percentages indicted very little due to the low patient numbers

Six percent of the patients were treated with **Cyclo/TNI** and twenty percent with **Cyclo/Alg.** They were all **SAA** transplants. Only one patient generated early responses in all three parameters. All the **SAA** allo**PBSCT** achieved a "12 month post trilineage response", however, none of the **SAA** allo**BMT** did. Unfortunately, the patient numbers were too low to establish any class of pattern.

In summary, following **Cyclo/TBI/TNI**, most patients still generated an early response with all three parameters. This indicated very little stromal damage. Although the medium term response was not as positive, this outcome possibly had nothing to do with preconditioning. Although only a small percentage of patients were preconditioned with

Bu/Cy, the erythrocytic line appeared to be more affected than the granulocytic or megakaryocytic line, but with very little medium term concern, as the achievement rate of "12 month post transplant trilineage response" was still less than fifty percent. The other preconditioning treatments, **Bu/Mel/Thiotep**, **Cyclo/TNI**, and **Cyclo/Alg** contained too few patient numbers for comment.

5.6. Mononuclear Count (MNC)

MNC were performed on all of the patients. Forty five percent of the patients produced counts above the mean (6.12 $x10^8$/kg), and forty one percent of them achieved a "12 month post transplant trilineage response". Of the patients with a higher **MNC**, about seventy percent generated early responses in all three parameters. Less than fifty percent achieved a "12 month post transplant trilineage response".

When subdivided, higher **MNC** were generated in seventy one percent of the allogeneic transplants and thirty six percent of the autologous transplants. Although only fifty percent of the allogeneic transplants generated an early **RMI** response compared to sixty percent **ANC** and **PLT**, less than thirty percent of them achieved a "12 month post transplant trilineage response". The autologous transplants generated a higher percentage of early **RMI** responses at sixty seven percent, with only a twenty five percent achieving a "12 month post transplant trilineage response". Only fifty percent of the **ANC** and sixty percent of the **PLT** autologous transplants generated early responses, one hundred percent of them achieved a "12 month post transplant trilineage response". It was clear that even with low patient numbers the autologous transplants with higher **MNC** generated earlier responses particularly in the **RMI** and **PLT** parameters, with a positive medium term prognosis. The allogenic transplants only produced earlier **ANC** responses in the patients with higher **MNC**.

Higher **MNC** were produced in forty three percent of the **PBSCT** patients, early **RMI** and **PLT** responses were seen in seventy percent of them and early **ANC** in sixty percent. The medium term prognosis was less positive with less than fifty percent of them achieving a "12 month post transplant trilineage response". Higher **MNC** were generated

in forty two percent of the **BMT** patients. Early responses were found in less than thirty percent of them, although almost one hundred percent achieved a "12 month post transplant trilineage response".

Higher **MNC** were found in forty six percent of the allo**PBSCT** patients. These resulted in early **ANC** and **PLT** responses in more than sixty percent of them. Less than forty five percent achieved a "12 month post transplant trilineage response". Only twenty nine percent of the auto**PBSCT** patients produced higher **MNC.** Fifty percent of them produced an early **RMI** response, but with no early **ANC** or **PLT** response. Only forty percent of them achieved a "12 month post transplant trilineage response". Higher **MNC** did result in earlier parameter responses particularly in the allo**PBSCT**, but with very little positive medium term success. Some higher **MNC** in the allo**PBSCT** indicated relapse. Four of the six patients with **MNC** count above 10 $x10^8$/kg relapsed. Even with low patient numbers in the auto**PBSCT**, it was obvious that higher **MNC** resulted in very few early responses, with little positive medium term success. The only difference was that more auto**PBSCT** achieved a "12 month post transplant trilineage response than the allo**PBSCT** regardless of **MNC.**

Within the smaller groups, the **AML** allo**PBSCT**, the **CML** allo**PBSCT** and the **AML** auto**PBSCT**, even with early responses in the three blood parameters, there were too few patient numbers for accurate assessment. However, a point worth mentioning, was that both the **AML** auto**PBSCT** generated early responses in all three parameters and achieved a "12 month post transplant trilineage response". In the **AML** allo**PBSCT** group, patients with **MNC** above the mean, generally, were the first to respond in all three parameters. The **CML** allo**PBSCT** with higher **MNC** resulted in no significant effect on Response Time in any of the three parameters. The **AML** auto**PBSCT** produced an earlier **RMI** response with a lower **MNC**, but not in any of the other parameters.

When **BMT** and **PBSCT** are separated in the "12 month post transplant trilineage response" group, the **BMT MNC** was lower at 1.22 $x10^8$/kg compared to the **PBSCT** mean of 6.84 $x10^8$/kg. Although medium term response was positive, only twenty

percent of the **BMT** and fifty one percent of the **PBSCT** with higher **MNC** produced early parameter responses.

In the auto**PBSCT** group, where fewer patients produced higher **MNC**, poor early responses were detected, but a positive medium term success was evident. A positive prognosis however, was evident in patients without early responses. The **BMT** also produced a low percentage of early responses, with most of them achieving a "12 month post transplant trilineage response". Unlike the auto**PBSCT** however, the early responses resulted in positive medium term success. The **BMT** possibly produced a superior quality of stem cells compared to the **PBSCT**, as the **BMT** who responded first, generated the highest count for the group. The **PBSCT** did appear to require higher counts to achieve a "12 month post transplant trilineage response". However, some of the higher counts in the **PBSCT** group relapsed. The **PBSCT** patients with results above 10 x^8/kg were not of the first to respond, quite the opposite, the majority of them relapsed or died. The **MNC** mean count for the "12 month post transplant trilineage response" group was 5.83 $x10^8$/kg. This unfortunately did not indicate that the successful transplants were the first to respond in any of the three blood parameters.

In the case of the "graft failures" the patient numbers were too low for comment, although, it did appear that the **MNC** was irrelevant. The first **SAA** allo**BMT** produced a low **MNC**, in both transplants. The second patient produced higher counts for the first transplant and low counts for the booster. The auto**PBSCT** produced a slightly low **MNC** and the **SAA** allo**PBSCT** produced a high **MNC** in both the transplant and the booster.

In conclusion, more than sixty five percent of the patients where the **MNC** exceeded the mean, early **RMI**, **ANC** and **PLT** responses were seen. The higher **MNC** and early responses did not appear to have any effect on the patients medium term prognosis, as less than fifty percent of them achieved a "12 month post transplant trilineage response".

5.7. Colony Forming Unit–Granulocytic Monocytic (CFU-GM)

CFU-GM counts were performed on eighty percent of the patients. Only thirty three percent produced counts greater than the mean (26.10 $x10^4$/kg). Of them thirty three percent achieved a "12 month post transplant trilineage response".

Of all the patients with higher **CFU-GM** counts, sixty seven percent of them produced early **RMI** and **ANC** responses. Seventy five percent of them generated an early **PLT** response indicating a relationship between **CFU-GM** counts and megakaryocytic recovery. Less than fifty percent of them achieved a "12 month post transplant trilineage response".

CFU-GM counts were performed on eighty percent of the allogeneic transplants. Only thirty nine percent of them produced **CFU-GM** counts above the mean. Although more than sixty percent generated early **RMI** and **ANC** responses, only fifty percent produced an early **PLT** response. The medium term prognosis was poor, particularly in the early **RMI** and **PLT** parameters where less than thirty five percent achieved a "12 month post transplant trilineage response". An improved prognosis was seen for early **ANC** response, fifty percent of them achieved a "12 month post transplant trilineage response". **CFU-GM** counts were performed on fifty percent of the autologous transplants. Only twenty five percent of them produced **CFU-GM** counts above than the mean. Even with low patient numbers all of them generated early **ANC** and **PLT** responses, but only half of them achieved a positive medium term success.

CFU–GM counts were performed on seventy four percent of the **PBSCT**. Only twenty nine percent of the counts were above the mean. Eighty percent of the patients generated early **ANC** and **PLT** responses, and sixty two percent an early **RMI** response. The medium term prognosis was poor at less than forty percent. Nine of the ten **BMT** had **CFU-GM** counts. Only one generated an early response in both the **RMI** and **ANC**. This patient achieved a "12 month post transplant trilineage response". Unfortunately no accurate assessment could be made due to the low patient numbers.

CFU-GM counts were performed on eighty three percent of the allo**PBSCT**. Only forty five percent of the counts were above the mean. Only forty four percent of the patients generated early **RMI** and **ANC** responses and all of them died. The **PLT** however, produced a seventy seven percent early response with twenty nine percent achieving a "12 month post transplant trilineage response". In a few cases, patients with counts above the mean did appear to generate earlier responses in all three parameters. Patients with results between 10 $x10^4$/kg and 60 $x10^4$/kg appeared to experience an improved chance of achieving a "12 month post transplant trilineage response". In the auto**PBSCT** fifty seven percent of the patients had **CFU-GM** counts, two of them with counts above the mean. Both generated early responses in the **ANC** and **PLT**, and one patient died. A higher percentage of early **PLT** responses were seen in the allo**PBSCT.** This indicated a better megakaryocytic response, particularly in patients with higher **CFU-GM** counts.

In the smaller groups, the **AML** allo**PBSCT** produced two **CFU-GM** counts above the mean. Both of them generated early responses in all three parameters, and a raised **MNC**, both of these patients died. The **CML** allo**PBSCT** also produced two patients with **CFU-GM** counts above the mean. They both produced early **PLT** responses. The one with an increased **MNC** survived. The **AML** auto**PBSCT** also produced two patients with **CFU-GM** counts above the mean; the one that survived had early responses in all three parameters and a raised **MNC**. The numbers were too low for accurate analysis, however, it was noted that all the patients with raised **CFU-GM** counts produced early **PLT** responses. They did not necessarily generate early **RMI** or **ANC** responses.

Within the "12 month post transplant trilineage response" group, only fifty percent of the patients produced **CFU-GM** counts above the mean (9.84 $x10^4$/kg). Both generated early **RMI** responses, neither of them produced an early **PLT** response. Of the **PBSCT**, seventy one percent had **CFU-GM** counts. Only two patients (twenty five percent) produced counts above the mean, and both produced early **PLT** responses.

The **CFU-GM** counts for the first **SAA** allo**BMT** were low in both transplants. The second **SAA** allo**BMT** had a **CFU-GM** count slightly below the mean following the first transplant, but higher than the mean following the second. The **AML** auto**PBSCT** had no **CFU-GM** count. This may have been directly related to the "graft failure"; although preconditioning may have been a contributing factor. The **SAA** allo**PBSCT** had a low **CFU-GM** count for the first transplant (6.60 $x10^4$/kg), and a high count for the second (34.80 $x10^4$/kg). The patient did achieve a successful "12 month post transplant trilineage response". It would not be pertinent to assume that the **CFU-GM** counts alone played any role in the "graft failures", however higher **CFU-GM** counts did appear to be related to engraftment.

Higher counts did not always indicate medium term success. This was evident as the "12 month post transplant trilineage response" group only had a mean **CFU-GM** count of 19.84 $x10^4$/kg. Of the eight **BMT** with **CFU-GM** counts, six were above 11 $x10^4$/kg, five relapsed and died, and two patients with early responses had no counts. Of the thirteen **PBSCT** patients with counts above 20 $x10^4$/kg, eight relapsed. One was a "graft failure" and four achieved a "12 month post transplant trilineage response". Two **AML** allo**PBSCT** were of the first to respond in all three parameters although both died. The two **CML** allo**PBSCT** were slower to respond.

Even with the discrepancies seen within the various publications only one (Neiderwiser *et al*,1983) found any correlation between number of nucleated cells transplanted and granulocyte recovery. Our study revealed that more than sixty five percent of patients with **CFU-GM** counts exceeding the mean, generated an early **RMI**, **ANC** and **PLT** response. The **PLT** was a particularly good responder with a seventy five percent early response, indicating a relationship between the **CFU-GM** count and megakaryocytic recovery. The medium term projection indicated that less than thirty eight percent of the patients with early responses would achieve a "12 month post transplant trilineage response".

5.8. Cluster Designation 34+ (CD34+)

Only seven patients in total had **CD34+** counts, all of them were **PBSCT**, with a mean of 83.83 (ranging from 27.82 to 247.28). Only two of the patients had counts above the mean, one died. The patient numbers were too low and the values too varied to draw any conclusions.

5.9. Response Range

5.9.1. Reticulocyte Maturation Index (RMI)

Twelve percent of the patients produced **RMI** values above **2SD** at least once during the first forty days post transplant. They were all **PBSCT**. Ten percent fell below **2SD**. Only one of the six patients with higher than **2SD RMI** values and two of the seven below, achieved a "12 month post transplant trilineage response".

In all of the cases where engraftment occurred, the established Response Range (Figure 9 Page 45) was of little significance. The most value was in determining the "graft failures". The first **SAA** allo**BMT RMI** values remained above the mean following the first transplant, but not in the second (an allo**PBSCT** transplant), where results ran below **2SD** until day twenty six, then rose above the mean prior to forty days. The **RMI** values for the second **SAA** allo**BMT** never reached the mean prior to the booster. A **RMI** response on day thirty seven was seen, indicating "graft failure". Following the booster, the **RMI** exceed the mean. The **AML** auto**PBSCT**, even with no Point of Response, maintained a **RMI** value within **2SD** for the first forty days even though the values remained below the mean. The **SAA** allo**PBSCT RMI** value remained below the mean following the first transplant, after the booster values above the mean were recorded.

In all transplant patients, **RMI** values should be monitored on a daily basis until recovery is established. Once a patient has accomplished erythrocyte recovery, **RMI** values above or below **2SD** are of little significance. Our aim was to establish whether values above **2SD** within the first forty days indicated medium term success. Unfortunately that was not possible due to the low patient numbers who generated higher **RMI** values and achieved a "12 month post transplant trilineage response".

In the case of suspected "graft failure" however, daily monitoring of **RMI** values would be pertinent. Not only because of a lack of a seventeen day Point of Response was observed, but because our research revealed that all the "graft failures" produced **RMI** values below the mean, following both the first and the second transplants.

5.9.2. Absolute Neutrophil Count (ANC)

Eighteen percent of the forty five patients produced **ANC** above **2SD** within the first forty days post transplant. Eight of the nine achieved a "12 month post transplant trilineage response" only one died of unrelated causes. Thirty nine percent fell below **2SD**; and six of the eighteen achieved a "12 month post transplant trilineage response".

Seven of the nine patients with **ANC** above **2SD** within the first forty days were autologous transplants. Six achieved a "12 month post transplant tri lineage response". The remaining two were **BMT** and both achieved a "12 month post transplant trilineage response". Not only did the autologous transplants produce higher **ANC** within the first forty days; but also more of them accomplished medium term successes. The only allogenic transplants with **ANC** higher than **2SD** were **BMT's**.

Of the eighteen patients with **ANC** below **2SD**, fourteen were allogenic transplants; and four achieved a "12 month post transplant trilineage response". The other four were autologous transplants. Three achieved a "12 month post transplant tri lineage response". Allogenic transplants did produce lower **ANC** following transplant, particularly in the first forty days.

The first **SAA** allo**BMT** **ANC** fell below the mean constantly from day ten following the first transplant. Following the second transplant, an **ANC** above the mean was seen from day twenty six until day forty. In the second **SAA** allo**BMT**, the **ANC** fell below the mean for the first thirty days. After the boost, although there was an improvement in **ANC** with counts above the mean, they were not as responsive as the **RMI**. The **AML** auto**PBSCT** **ANC** failed to reach the mean at any point within the first forty days. The **SAA** allo**PBSCT** **ANC** following the first transplant not only failed to reach the mean, but never achieved a Point of Response, indicating "graft failure". Following the booster

an **ANC** Point of Response was indicated within ten days. These counts stabilised and remained above **2SD** until the patient was released. The **ANC**, like the **RMI**, was an important parameter for monitoring recovery for the first forty days, particularly to sift out "graft failure" and to establish engraftment.

ANC is a routinely monitored parameter post transplant. In our patients, most of them with counts exceeding **2SD**, were autologous transplants with all but one achieving a "12 month post transplant trilineage response". Although the autologous transplants do appear to produce higher **ANC**, any patient with counts exceeding **2SD**, lacking clinical infection within the first forty days stands an excellent chance of engrafting and achieving medium term success.

5.9.3. Platelet Count (PLT)

Because the **PLT** was the most unstable parameter of all three, counts above **2SD** were seen in all the patients at one point during the first forty days. All the patients produced **PLT** below 10 x10^9/l at some point.

The first **SAA** allo**BMT** produced low **PLT** for both transplants, with counts below the mean for most of the first forty days. Following the second transplant, the **PLT** fluctuated. Dramatic effects were seen following twenty six days, with a considerable decrease in counts reaching numbers below the mean. The second **SAA** allo**BMT** produced **PLT** below the mean most of the time. The **AML** auto**PBSCT** occasionally produced **PLT** above 50 x10^9/l otherwise they remained below the mean. In the **SAA** allo**PBSCT**, the **PLT** did not indicate "graft failure". Following the first transplant, counts remained below the mean for most of the forty days. Following the booster, **PLT** above the mean and **2SD** were seen.

The **PLT** was the most unstable of all three parameters. Although as an indicator of megakaryocytic recovery, the **PLT** was an accurate parameter. Unlike the **RMI** and **ANC**, the **PLT** was not an ideal tool for predicting "graft failure" or positive medium term success.

5.10. "Day 14" Marrow Biopsy

Only thirty nine percent of the patients received "day 14" marrow biopsies. Of those that revealed a trilineage response on "day 14", one hundred percent produced the same response on "day 28". "Day 14" marrow biopsies revealed nothing additional compared to the "day 28" marrow biopsies.

However, a few cases are worth mentioning. These indicate the importance of peripheral blood counts being monitored parallel to marrow biopsies:

- One **AML** allo**PBSCT** with no erythroid and megakaryocytic engraftment at "day 28", might have benefited from a "day 14" marrow. The peripheral blood counts however, indicated the opposite. The **RMI** Point of Response was day one, and both the **ANC** and **PLT** on day eight. The **RMI** and **ANC** continued to rise for the first forty days, whereas the **PLT** decreased rapidly. Perhaps the marrow biopsy was taken from a poor proliferative area.

- The first **SAA** allo**BMT** received "day 14" marrow biopsies for both transplants. However, the "day 14" marrow revealed nothing more compared to the "day 28" marrow biopsy. Following the first transplant, "graft failure" was indicated on the "day 14" marrow and in the peripheral blood parameters, where the **ANC** failed to recover. The **RMI** recovered and then decreased, while the **PLT** fluctuated the entire time. Following the **PBSCT**, both the "day 14" and "day 28" marrow showed trilineage engraftment, whereas, the **RMI** failed to recover and indicated a "graft failure".

- In the second **SAA** allo**BMT** both the "day 14" marrow and peripheral blood parameters indicated "graft failure". All three peripheral blood parameters revealed counts below the mean. Both the peripheral blood parameters and "day 28" marrow indicated a good granulocytic and erythroid recovery with no evidence of megakaryocytic recovery. The **RMI** exceeded **2SD**, but the **ANC** never reached the mean, and the **PLT** never indicated engraftment.

- In the **AML** auto**PBSCT** "graft failure", the peripheral blood parameters and the "day 14" marrow results were the same, as the patient was aplastic. Although the "day 28" marrow suggested a small amount of granulocytic response, the **RMI** and **ANC** indicated "graft failure". Subsequent marrow biopsies revealed no engraftment.

- An **AML** auto**PBSCT** with delayed **ANC** and **PLT** peripheral blood counts, did however, have a trilineage response on both the "day 14" and "day 28" marrow biopsies.

Our findings indicated that the "day 14" marrow biopsy revealed nothing that the "day 28" marrow biopsy did not. It was however important to take into consideration the **RMI, ANC** and **PLT** peripheral blood parameters as additional indicators of engraftment or "graft failure".

All of the transplant patients in this study received T-Cell depleted transplants. Although the **ANC** was a very sensitive indicator of transplantation, if the counts dropped due to infection, it could lead to a false impression of transplant failure. Particularly in allogeneic transplants as a result of **G-VHD** (Davis *et al*,1989). The **RMI** was not sensitive to infection making it a very good indicator for engraftment. If all three parameters were used in combination with marrow biopsies, there would be a decrease in patient pain and cost incurred from repeated procedures.

CHAPTER 6

Conclusion

The Reticulocyte Maturation Index (**RMI**) proved to be an excellent early indicator not only for erythrocytic recovery in bone marrow and peripheral stem cell transplants, but as an indicator for engraftment in all three cell lines, erythrocytic, granulocytic and megakaryocytic. Of the eleven groups analysed, the **RMI** mean Response Time of eight days was earlier than observed in most publications (Batjer *et al*,1994;Dahal *et al*,1996; *Davis et al*,1992;d'Onofrio *et al*,1996;Greinix *et al*,1994). The **RMI** was not only the first blood parameter to recover in all the transplant patients, but was the most stable, maintaining values of greater than 0.20 IU for the first forty days following recovery (Graph 1 Page 49). The **RMI** mean Response Time had no medium term prediction value, although it did indicate that the marrow needed to generate a healthy recovery within forty days.

Our research findings revealed that bone marrow and peripheral blood stem cell transplants should achieve **RMI** values of 0.20 IU or more within seventeen days post infusion to be considered a successful engraftment. If an **RMI** value of 0.20 UI has not been achieved or failed to remain within the normal range (0.20 IU – 0.50 IU) for any length of time, "graft failure" should be a consideration. A normal **RMI** value was expected to rise and reach a mean of approximately 0.39 IU (a range of 0.33 IU to 0.45 IU) within forty days. This depended on which type of transplant was received. The **RMI** decreased and fell to levels below normal within six months.

The Absolute Neutrophil Count (**ANC**) mean Response Time was found to be an excellent tool for assessing granulocytic recovery. Our mean Response Time of between thirteen and twenty days, depending on transplant type, was in agreement with other publications (Batjer *et al*, 1994; Dahal *et al*, 1996; Davis *et al*, 1989&1992; d'Onofrio *et*

al, 1996; Greinix *et al*, 1994; Testa *et al*, 1997). Bone Marrow Transplants (**BMT**) were found to have a slow response, but produced superior counts within forty days. This remained stable for six months. Peripheral Blood Stem Cell Transplants (**PBSCT**), when divided, proved that allogenic Peripheral Blood Stem Cell Transplants (allo**PBSCT**) were the earliest responders, while the Chronic Myeloid allogenic Peripheral blood Stem Cell Transplants (**CML** allo**PBSCT**) were the exception retaining the slowest mean Response Time. The autologous Peripheral Blood Stem Cell Transplant (auto**PBSCT**) produced slower responders but superior **ANC** at forty days and six months. Seventy seven percent achieved a "12 month post transplant trilineage response".

Other investigations (Davis *et al*, 1989; Chin-yee *et al*, 1991; Greinix *et al*, 1994; d'Onofrio *et al*, 1996) agreed that if an **ANC** has not reached 0.50 $x10^9/l$ within twenty eight days, "primary graft failure" should be considered. In conjunction with our research, our opinion is that this needs to be reevaluated. An **ANC** response should be seen within at least thirty two days before "primary graft failure" is considered. An **ANC** was expected to reach a mean of approximately 1.63 $x10^9/l$ within forty days, with a further increase to 2.77 $x10^9/l$ within six months. These counts were however found to be group dependent.

The **ANC** Point of Response allowed for the creation of confidence limits within the various groups to be analysed. More **PBSCT** patients responded within a shorter time frame than **BMT** patients. This strengthened the belief that **PBSCT** are superior **ANC** responders. The auto**PBSCT** revealed that more patients responded within a shorter period of time compared to allo**PBSCTS.** This confirmed a "pattern of recognition" within autologous transplants, allowing them to produce a higher percentage of patients achieving a positive medium term prognosis.

The Platelet Count (**PLT**) was found to be the least stable of the three blood parameters. Although the **PLT** mean Response Time proved a worthy estimation of megakaryocytic recovery, the fluctuations in counts rendered them unreliable. Our research findings lead us to believe that a **PLT** response of 50 $x10^9/l$ should be seen within thirty six days. A

mean count of approximately 60 $x10^9$/l should be reached within forty days, resulting in a count of about 141 $x10^9$/l at six months. These counts were group dependent. The **PLT** Point of Response did not reveal as much information as the **RMI** or **ANC**, but did indicate a "pattern of recognition" within the autologous transplants.

Patients preconditioned with **Cyclo/TBI/TNI** produced approximately sixty percent early responses in all three parameters. Although auto**PBSCT** produced higher percentages of early **RMI** responses, the **ANC** and **PLT** produced higher early responses within the allo**PBSCT**. Because more than fifty percent of the patients generated an early response in all three blood parameters, the likelihood of stromal damage was minimal. All the patients preconditioned with **Bu/Cy** were allogenic transplants. Less than thirty percent of them produced early **RMI** responses, however more than forty percent revealed an early **ANC** and **PLT** response. It was possible that Bu/Cy caused more stromal damage than **Cyclo/TBI/TNI.** Perhaps the type of preconditioning used, was a direct link to why less **PBSCT** achieved a "12 month post transplant trilineage response". The other preconditioning treatments contained too few patient numbers for comment.

In more than sixty five percent of the patients where the **MNC** was greater than the mean, early **RMI**, **ANC** and **PLT** were observed. This led to the conclusion that higher counts do produce earlier responses. Lower percentages of patients generated earlier **RMI** responses in allogenic transplants when compared to autologous transplants. Unfortunately, higher **MNC** revealed no evidence to support the belief that these counts had any positive projection value on medium term success within the transplants.

More than sixty five percent of the patients with higher **CFU-GM** counts than the mean, generated early responses within the **RMI**, **ANC** and **PLT** parameters. Although allogenic transplants produced more early **RMI** responses than the other two, both the autologous transplants and the allo**PBSCT** generated a far higher percentage of early **PLT** responses. These research findings revealed a definite relationship between higher **CFU-GM** and early megakaryocytic recovery. Unfortunately like the **MNC**, higher **CFU-GM** counts divulged no positive projection for medium term success.

The determination of a Response Range for the first forty days proved to be valuable. All patients' peripheral blood counts should be monitored on a daily basis at least until engraftment has occurred. Although the **RMI** parameter was found to produce very few values greater than **2SD** within the first forty days, monitoring of patients was particularly important in the case of "graft failures" as values below the normal range were detected throughout most of the forty day period. The **ANC** however, proved that where counts greater than **2SD** were produced, and infection was ruled out, there was a chance of achieving a"12 month post transplant trilineage response". The **PLT** was unreliable because it was unstable with most of the patients producing counts greater than **2SD** within the first forty days, as well as decreasing below 10 $x10^9$/l.

None of the "day 14" marrow biopsies added any information that the "day 28" marrow biopsy did not. If the "day 14" marrow biopsy procedure was to be discontinued, the importance of reliable peripheral blood counts to monitor patient progress becomes increasingly important. If all three parameters **RMI**, **ANC** and **PLT** were readily available, it would not only be cost effective, but would protect the patient from painful and uncomfortable procedures early in the post transplant period. In a few cases our research found that where the biopsies proved to be inadequate, the three peripheral blood parameters were valuable in assessing bone marrow production and therefore engraftment.

In conclusion, the **RMI** proved to be an invaluable parameter when monitoring both **BMT** and **PBSCT**. Not only is it a valuable tool for engraftment, but also for monitoring post transplant recovery. The reason for this is that it plays an integral role in the determination of "graft failure" prior to the **ANC** or "day 28" marrow biopsy.

FURTHER STUDIES

We have established an expected Point of Response for the Reticulocyte Maturation Index (**RMI**) in post allogenic Bone Marrow Transplant (allo**BMT**), allogenic Peripheral Blood Stem Cell Transplant (allo**PBSCT**) and autologous Peripheral Blood Stem Cell Transplant (auto**PBSCT**) patients. It is clear at this point that if no **RMI** response is found within seventeen days the likelihood of "graft failure" is altitudinous. This was proved in the two Severe Aplastic Anaemia allogenic Bone Marrow Transplant (**SAA** allo**BMT**), the Acute Myeloid Leukaemia autologous Peripheral Blood Stem Cell Transplant (**AML** auto**PBSCT**) and the Severe Aplastic Anaemia allogenic Peripheral Blood Transplant (**SAA** allo**PBSCT**) "graft failures". In addition all the patients who engrafted, achieved an **RMI** Point of Response within seventeen days.

At the time of the study most of the acute leukaemias were admitted to the Department of Haematology, Groote Schuur Hospital on presentation of disease. Their **RMI** levels were monitored throughout the entire treatment process and post transplant period. A considerable number of patients who died prior to transplant were monitored as well.

We are hoping to examine all these results with the intention of establishing an **RMI** Point of Response as an indicator to successful pre transplant treatment, and establishing pre transplant failure with the same accuracy detected in failed responses post transplant.

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