DONOR CHIMERISM ANALYSIS IN PATIENTS OF HEAMATOLOGICAL DISEASES AFTER BONE MARROW TRANSPLANT

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CHIMERISM

- In Greek - Eygptian mythology
  - **A composite of a lion, goat and serpent**
  - **The Minoan Minotaur from Crete combined a man and a bull**
  - **Sphinx – lion's body and man's head**

- Chimera
  - **An animal or human being with two or more different populations of genetically distinct cells**

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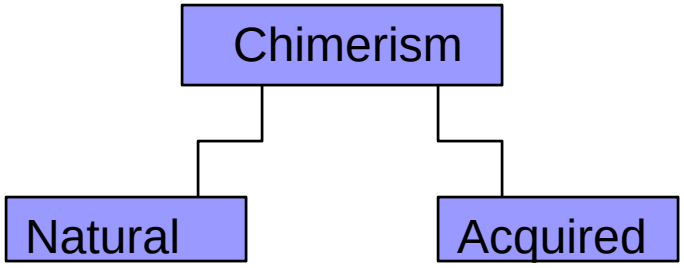
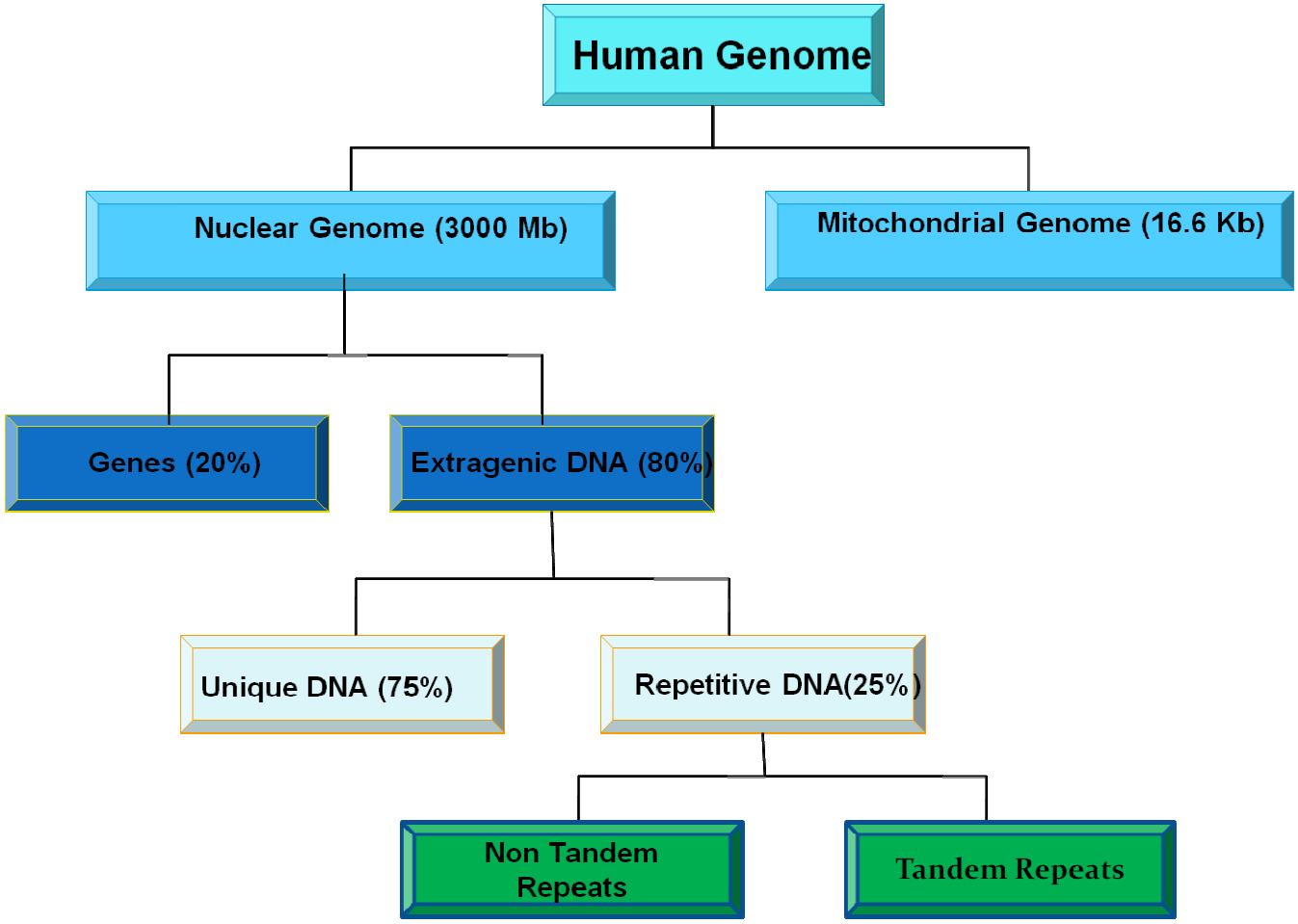


CHIMERISM IN BONE MARROW TRANSPLANT

- Complete donor chimerism
- Mixed donor chimerism
  - Increasing/progressive
  - Stable
  - Decreasing/reducing
- Testing for Donor chimerism
  - Variable number of tandem repeats
  - Short tandem repeats
  - Single nucleotide polymorphism
  - CD34+ cell sorting

SHORT TANDEM REPEATS(STRs)



SHORT TANDEM REPEATS

- Sequences of 2 – 10 base pairs
- Different number of repeats in different individuals
- 4 – 50 tandem repeats

AGCCTTTAAGCCTACCAGTCATTCCATTATGATG
AAAAGGTGGCCAATGTGCTGCTGACTGACTGACTGACTGAC
TGACTGACTGACTGACTGACTGACTGAAAGTCTTGAACGG
TCGATCGAATCGGGTACTCTAGGAA

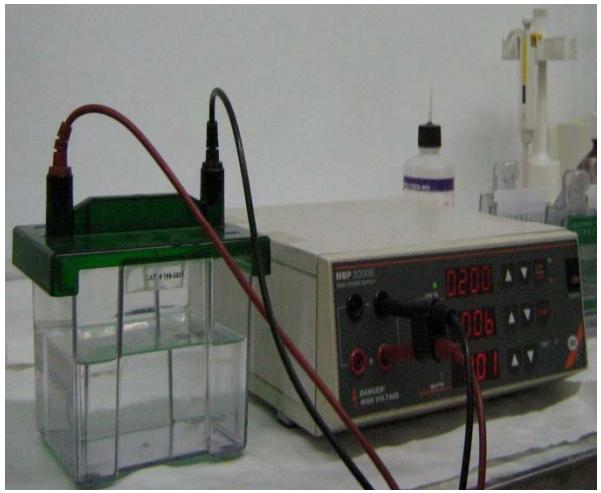
ANALYSIS OF SHORT TANDEM REPEATS

STR markers used

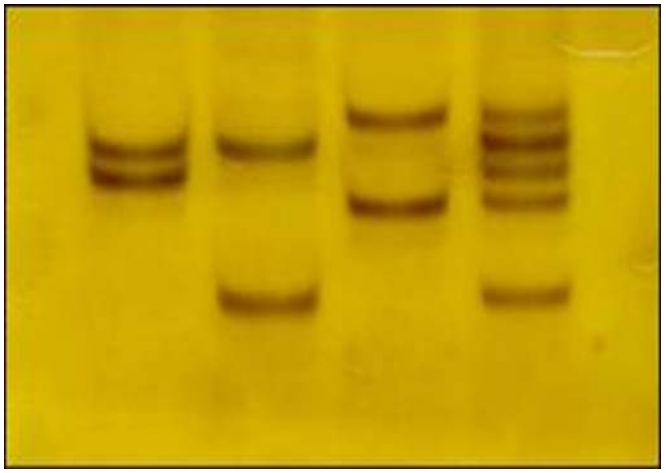
- D3S1358
- D5S818
- D7S820
- D8S1179
- D13S317
- D21S11
- TPOX
- FGA
- Th01

- **Manual method**
- **Automated method**

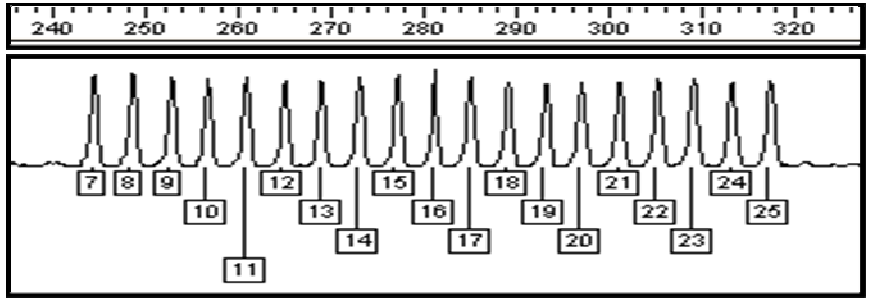
Thermal cycler

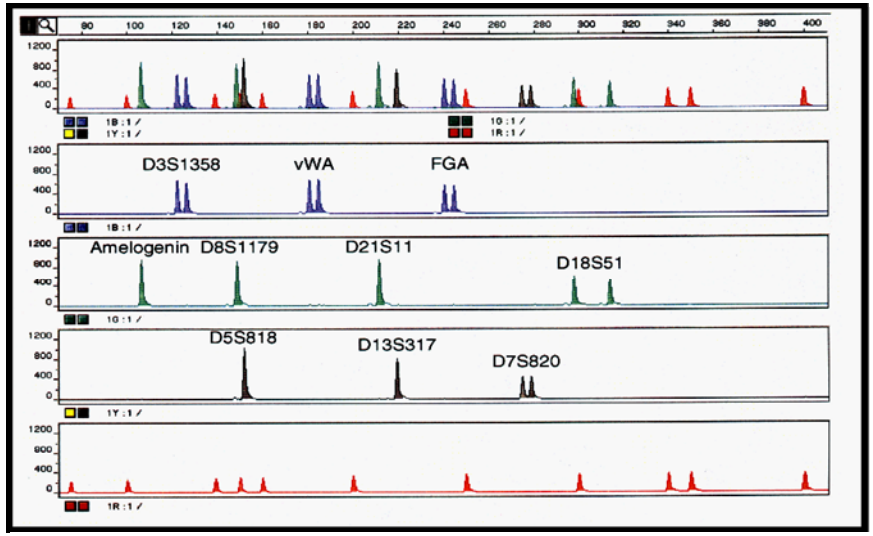


Electrophoresis equipment



Gel electrophoresis





Results from automated method

Applications of Chimerism Analysis

- Correlation with graft versus host disease

- Prognostic indicator, to predict:
  - Engraftment (bone marrow & organ transplant)
  - Relapse/rejection

THE STUDY

- Objective
  - To study the frequency of donor chimerism in patients of haematological diseases after bone marrow transplant
- Study design
  - Cross-sectional study
- Setting and Duration
  - Haematology department, Armed forces Institute of Pathology
  - July 2009 – January 2010

THE STUDY

- Patients and Methods
  - 57 patients
  - Beta thalassaemia major, Aplastic anaemia, Acute leukemia, Chronic myeloid leukemia, lymphoma
  - HLA matched bone marrow transplant
  - Between 3 to 6 months ago

THE STUDY

- Patients and Methods(contd)
  - Samples taken – buccal smear, peripheral blood, bone marrow
  - Chimerism assessed by STR-PCR analysis
  - Haematological parameters were done using Sysmex KX-21

THE STUDY

- Results
- Age

<10yr	10-14yr	15-19yr	20-24yr	25-29yr	30-34yr	35-39yr	>40yr
32(56.1%)	11(19.2%)	5(8.7%)	2(3.5%)	3(5.2%)	2(3.5%)	1(1.7%)	1(1.7%)

- Gender

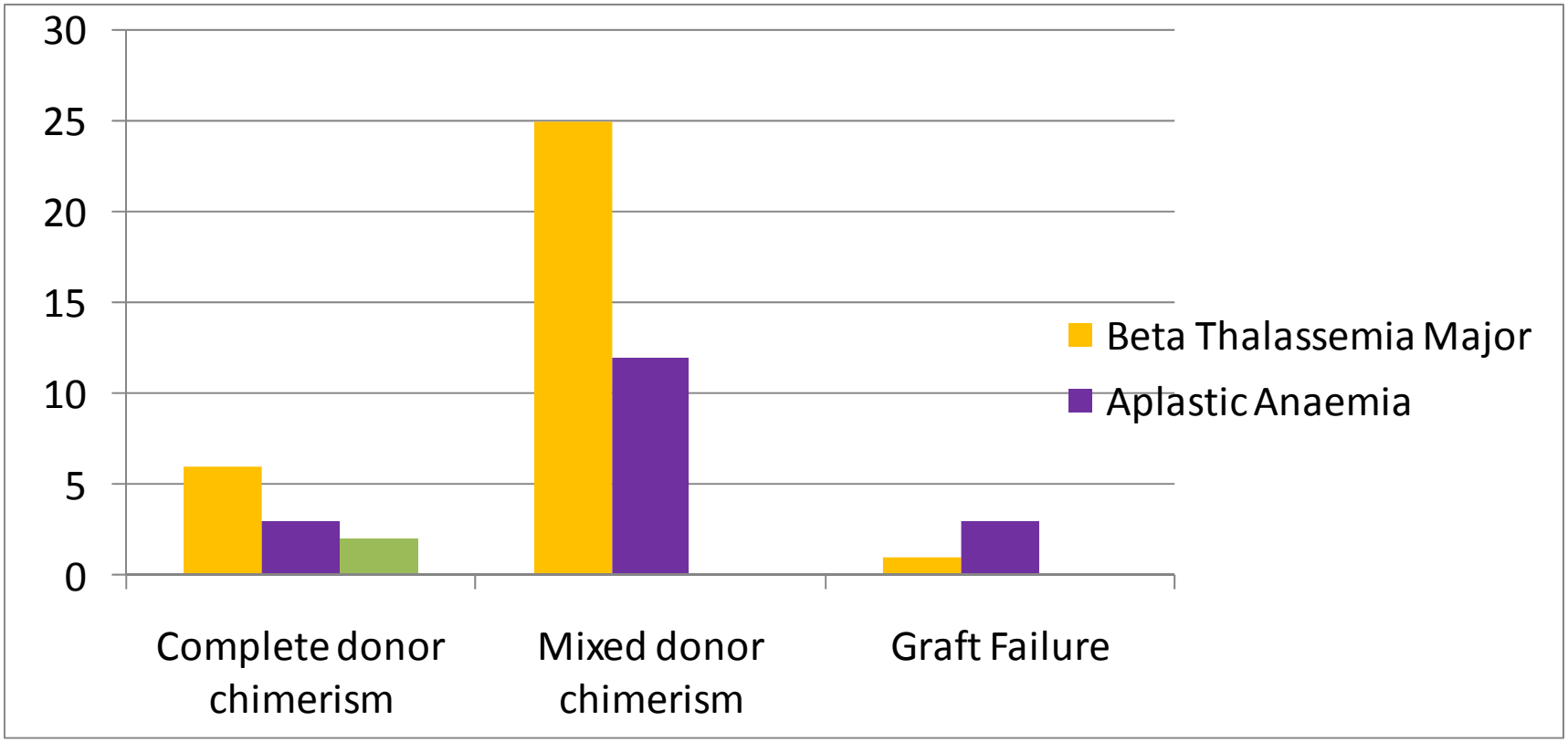
Male	Female
41(71.9%)	16(28.1%)

- Diseases

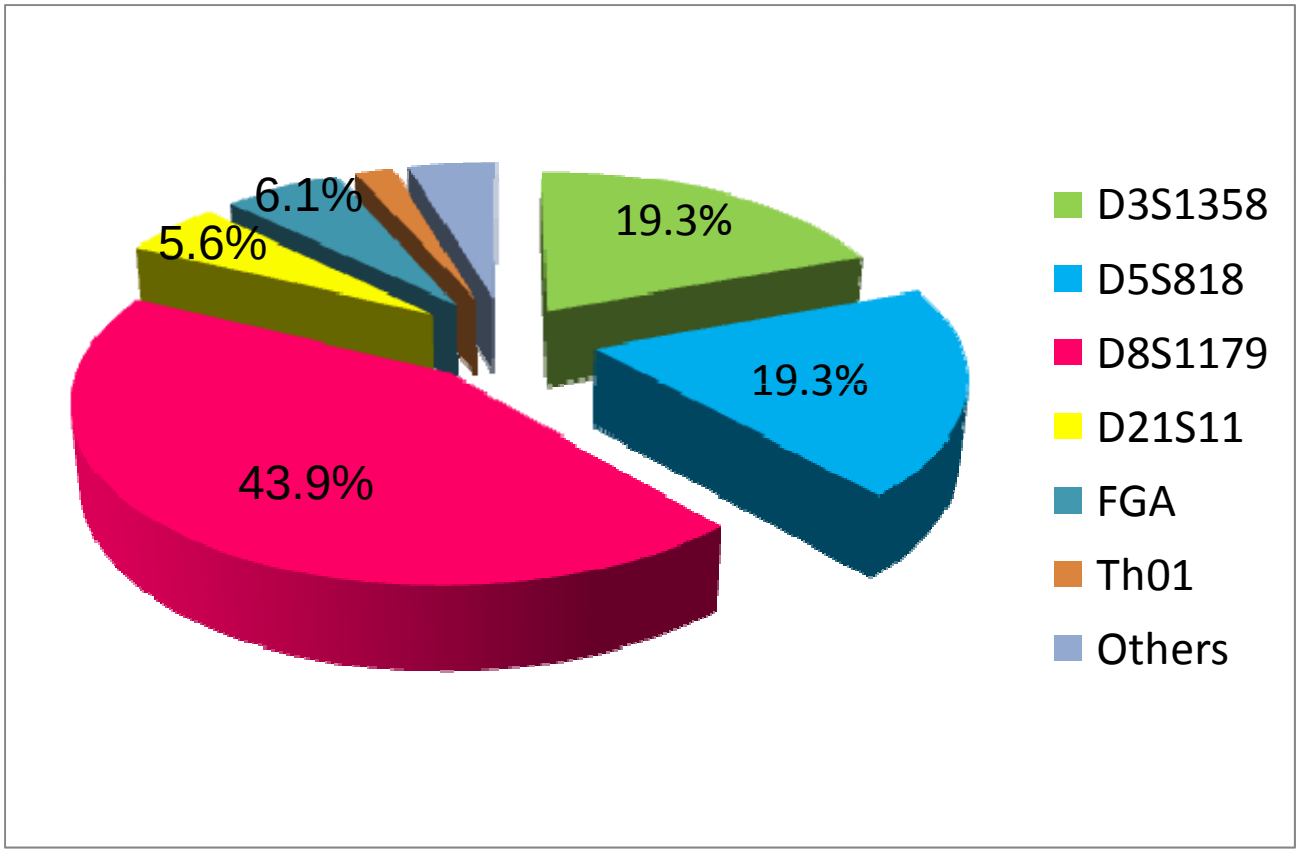
Beta thalassaemia major	Aplastic anaemia	Acute leukemia	Chronic myeloid leukemia	Lymphoma
32 (56.1%)	18(31.6%)	2 (3.5%)	3 (5.2%)	2 (3.5%)

- Chimerism Status

Complete Donor Chimerism	Mixed Donor Chimerism	Graft Failure
11 (19.2%)	42 (73.6%)	4 (7.2%)


30
25
20
15
10
5
0
Beta Thalassemia Major
Aplastic Anaemia
Complete donor chimerism
Mixed donor chimerism
Graft Failure

- Frequency of STR markers used

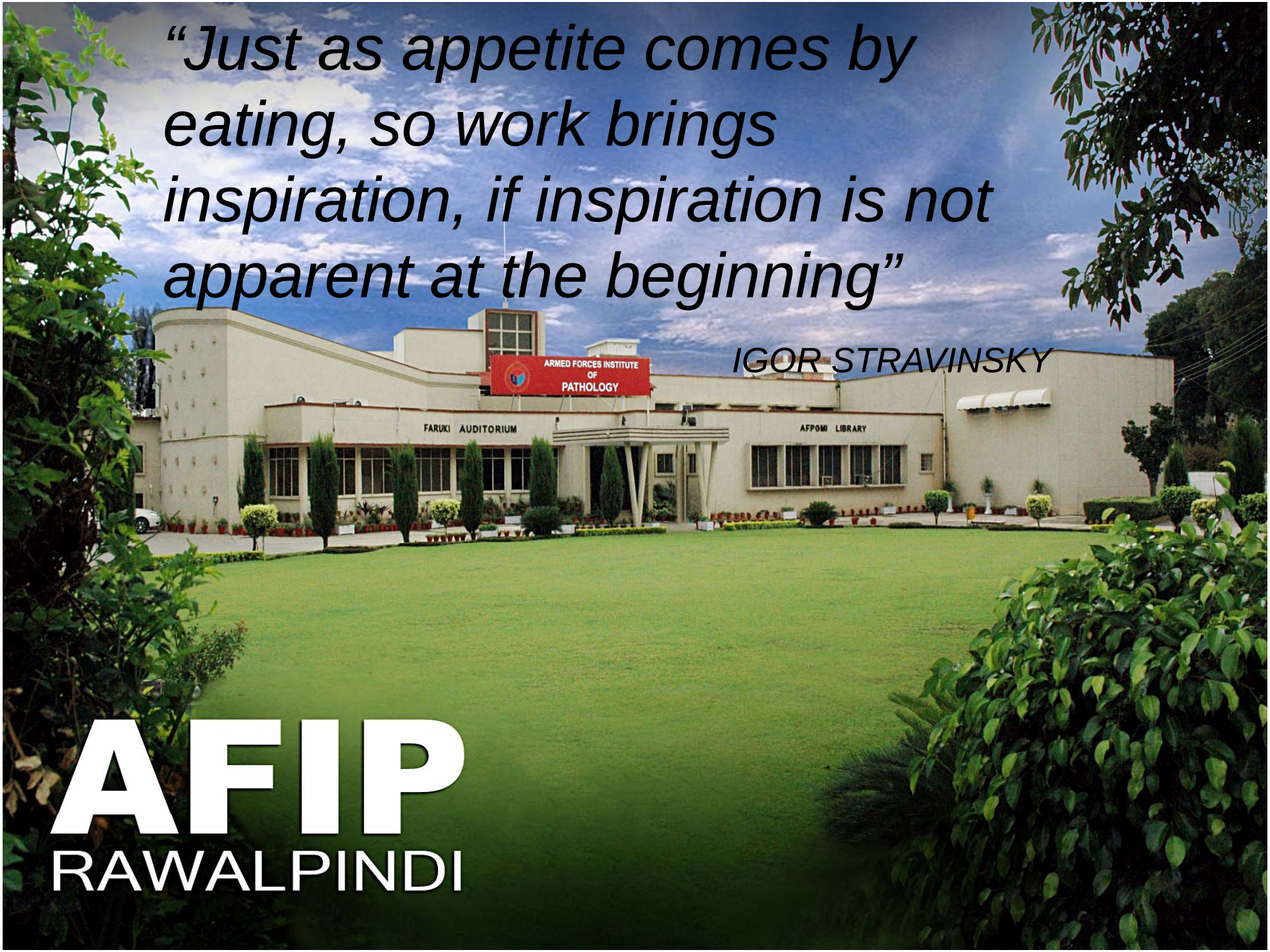


Correlation with Complete blood counts

- Haematological Parameters
  - Haemoglobin
  - Total leukocyte count
  - Absolute neutrophil count
  - Platelets

Conclusion

- Quantitative chimerism analysis provides valuable information regarding degree of donor haematopoiesis in the recipient. It can useful in serial follow up of transplant patients and in making decisions with regard to retransplant, donor lymphocyte infusions and other therapies.


"Just as appetite comes by eating, so work brings inspiration, if inspiration is not apparent at the beginning"
IGOR STRAVINSKY
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