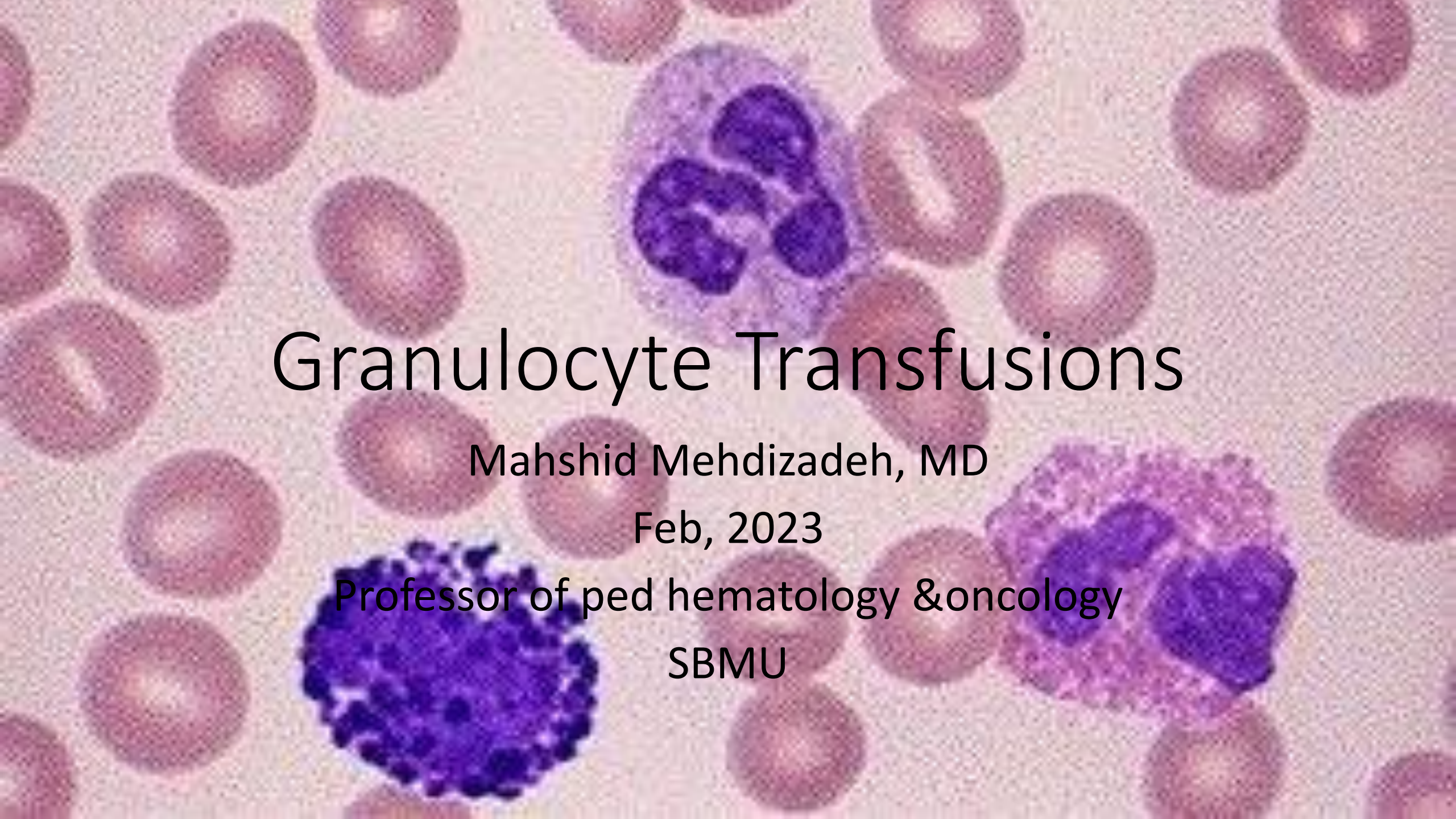

# Granulocyte Transfusions

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Feb, 2023

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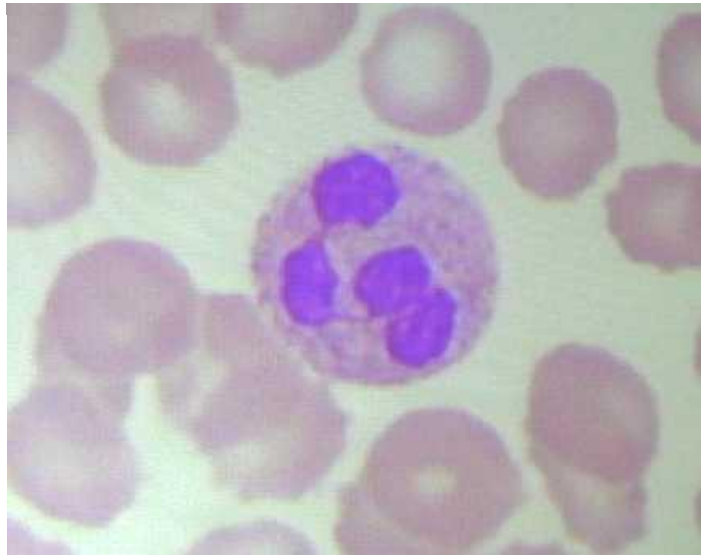
# Neutrophils

- Neutrophils
  - an essential component of the innate immune response
  - the first line of defense
- granulocyte transfusions are infrequently used in practice
  - paucity of supporting evidence of efficacy
  - potential adverse effects
  - limited availability of high-quality products.

# Granulocyte Transfusion : Historical Background

- Performed in 1960s using donors with CML
- 1985-1995: less interest due to equivocation of evidence, poor/time-consuming collections
- Resurgence of interest:
  - improved collection methods with instrumentation and pharmacologic agents
  - Anti-microbial resistance



Morse et al. (1966)

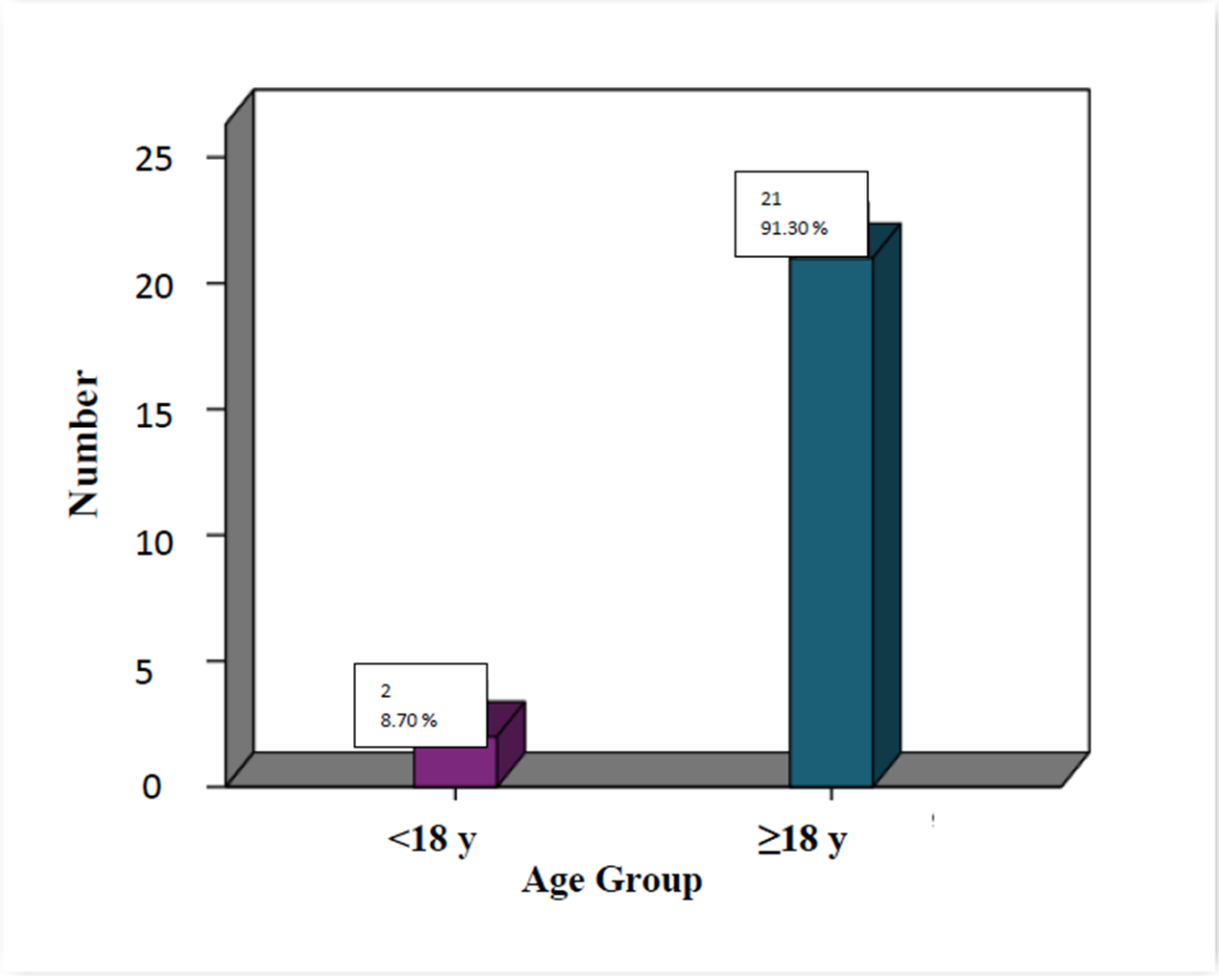
# Results of patients with granulocyte injection between 1390 and 1400

- From 1390 to 1400 33 patients with prolonged severe febrile neutropenia received Granulocytes  transfusion,
- 26 out of them were under HSCT
- Granulocytes were collected from healthy family iso blood group donors
- all donors had complete  viral andonly viable for 24 hours
- At least 4 × 1010 neu- trophils  up to 10.5  x 10 10 (mean 8 x 1010) Granulocytes harvested  by optia cell separator
- Donor received  prednisolone 50 mg po daily +/- GCSF 300 microgram SQ x 2 days
- Side effects:
- No transfusion reaction

| Adverse effect | Number of events |
| --- | --- |
| Thrombocytopenia (PLT count. <100 × $10^9$/L) | 4 |
| Venous access problems. | 0 |
| Numbness. | 6 |
| Bone pain | 13 |
| Head ache | 10 |
| Myalgia | 4 |

# Demographic Result

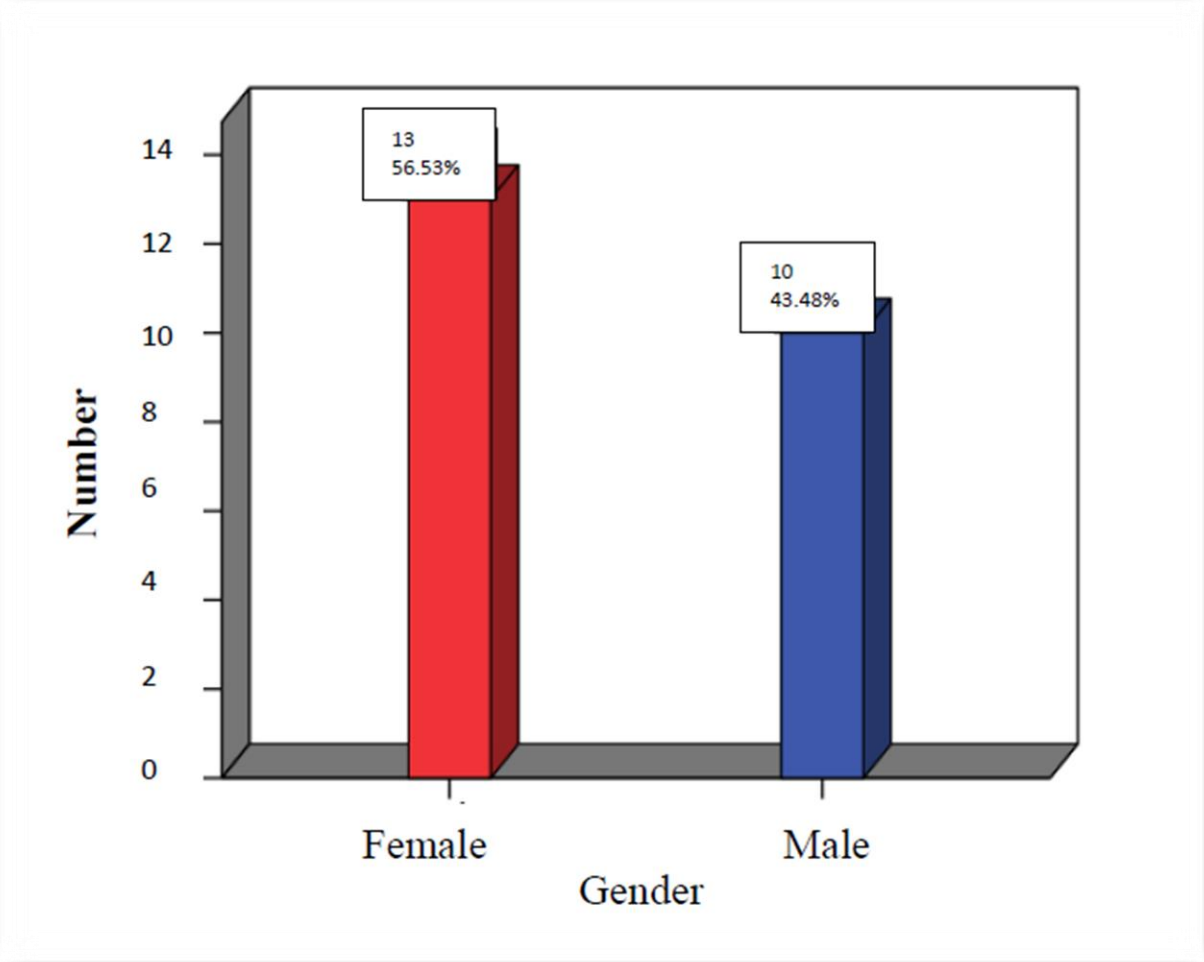
## Age




Out of 26 patients who received granulocytes, 2 Patients < 18 years (8.7 %) were in the age group under 18 years, and 21 Patients ≥ 18 years (91.3%) were in the age group equal to and older than 18.

## Demographic Result

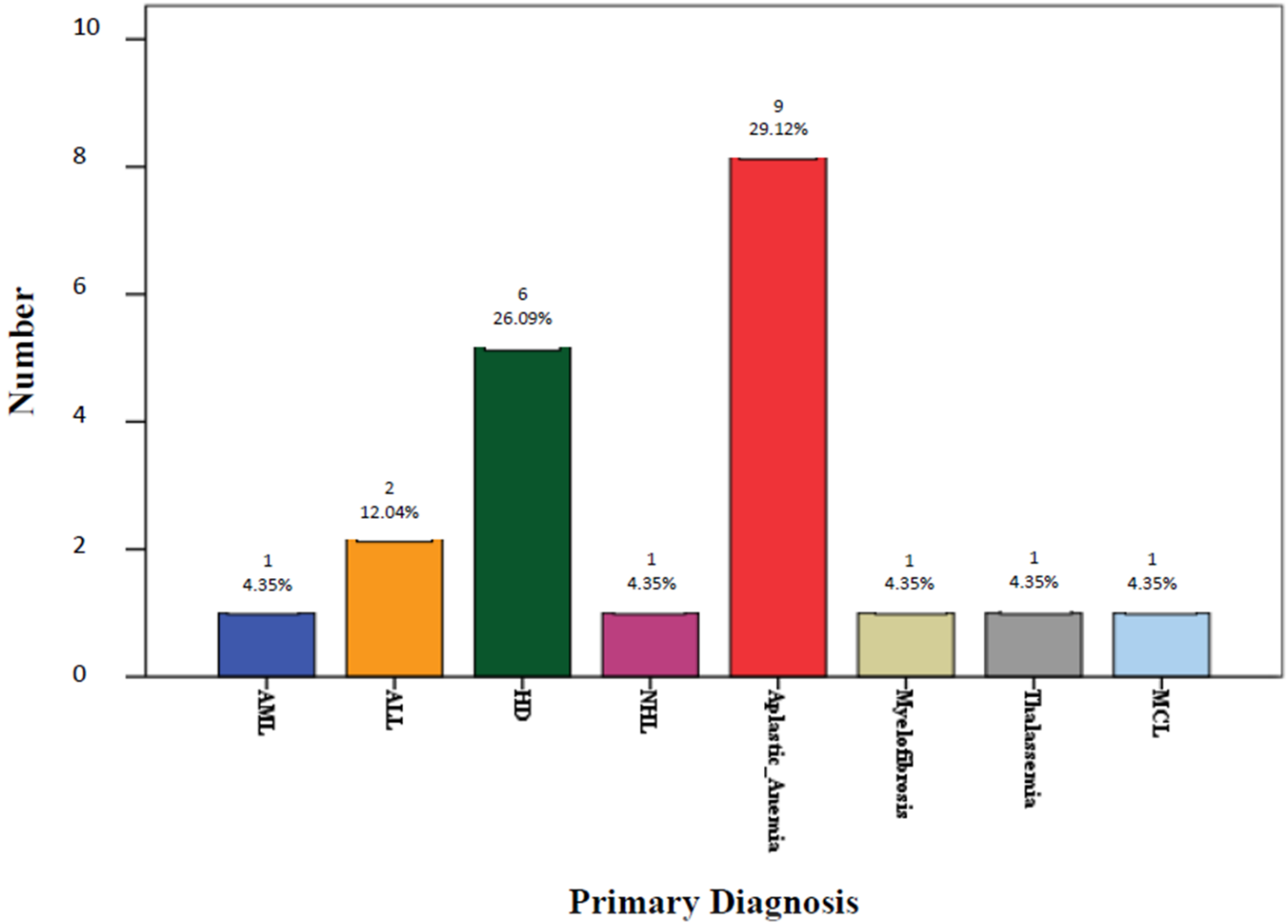
## Gender




Out of 26 patients who received granulocytes 13 Patients (56.53 %) were Female, and 10 (43.48%) were Male.

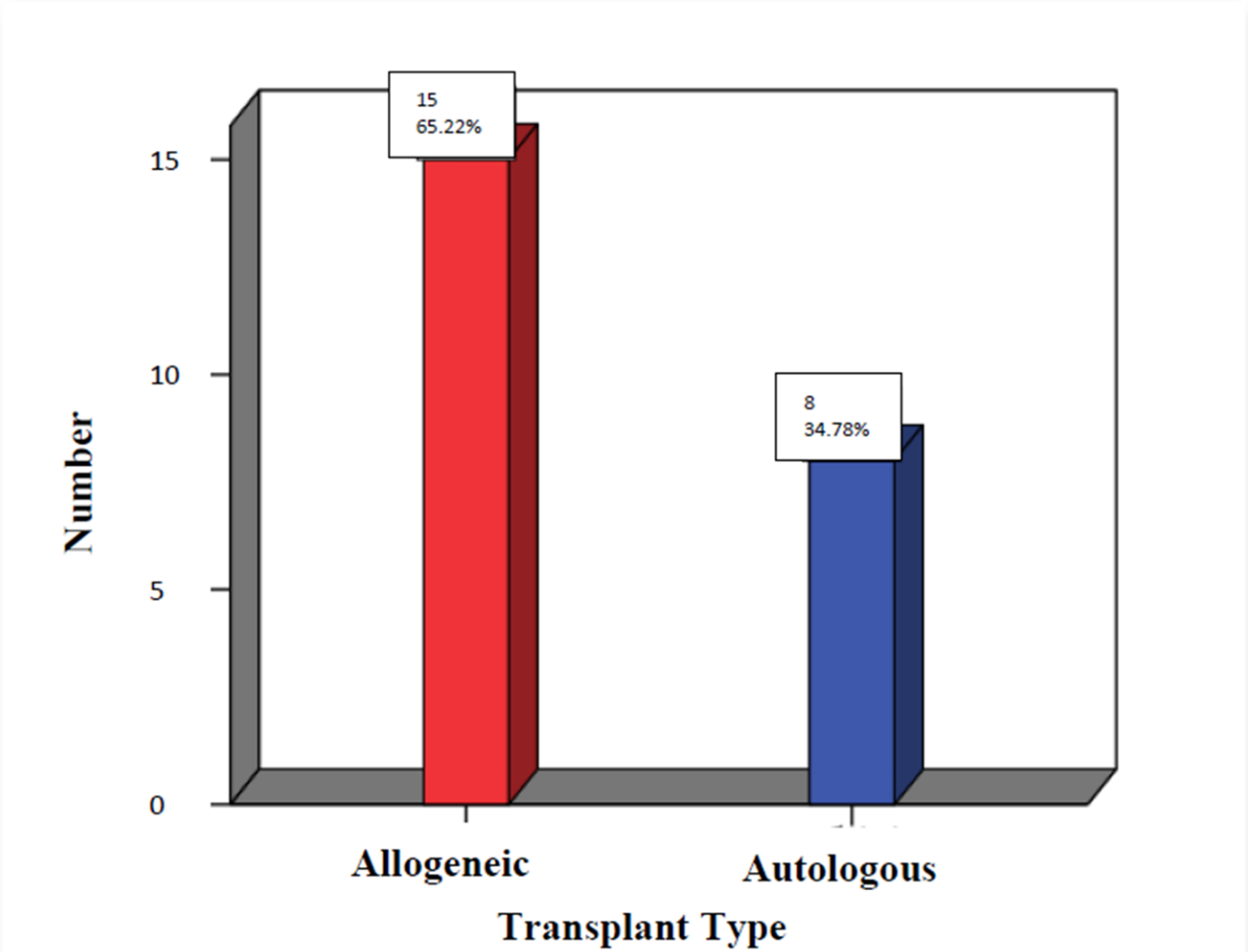
Demographic Result

Primary Diagnosis



## Demographic Result

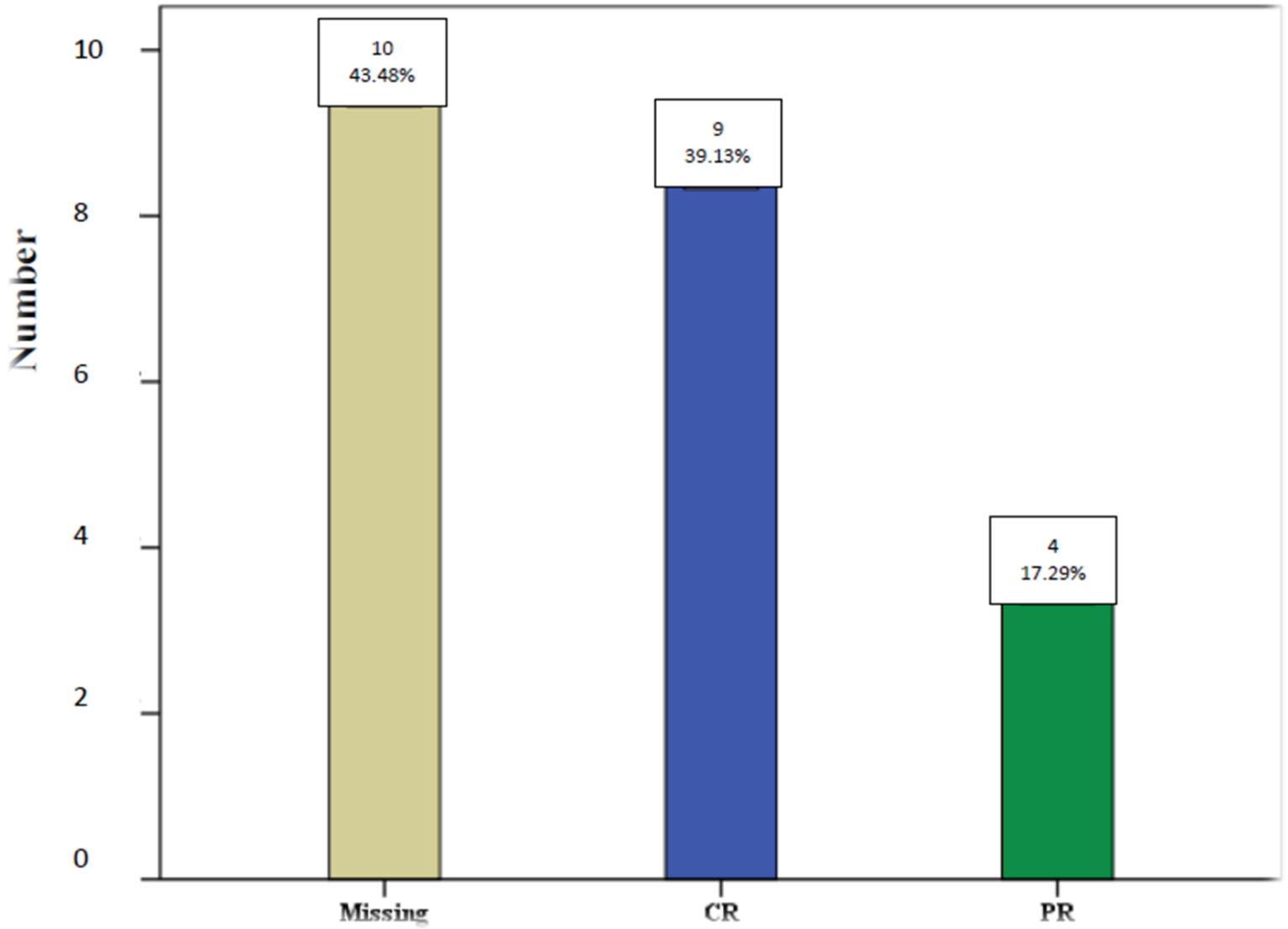
## Transplant Type




Out of 26 patients who received granulocytes 15 Patients (65.22 %) were undergoing Allogeneic HSCT, and 8 (34.78%) were undergoing Autologous HSCT.

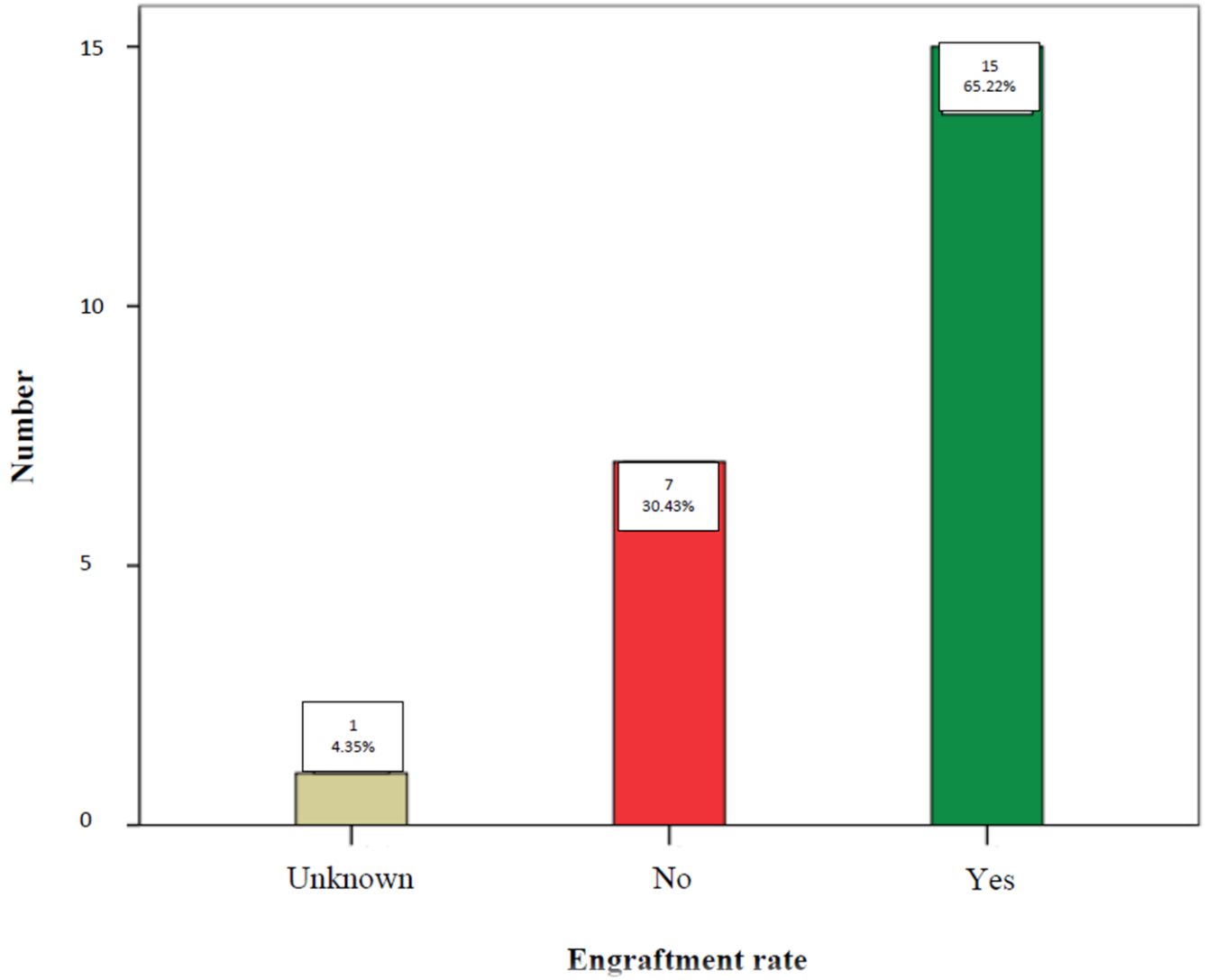
Clinical Features

Last Status Before HSCT



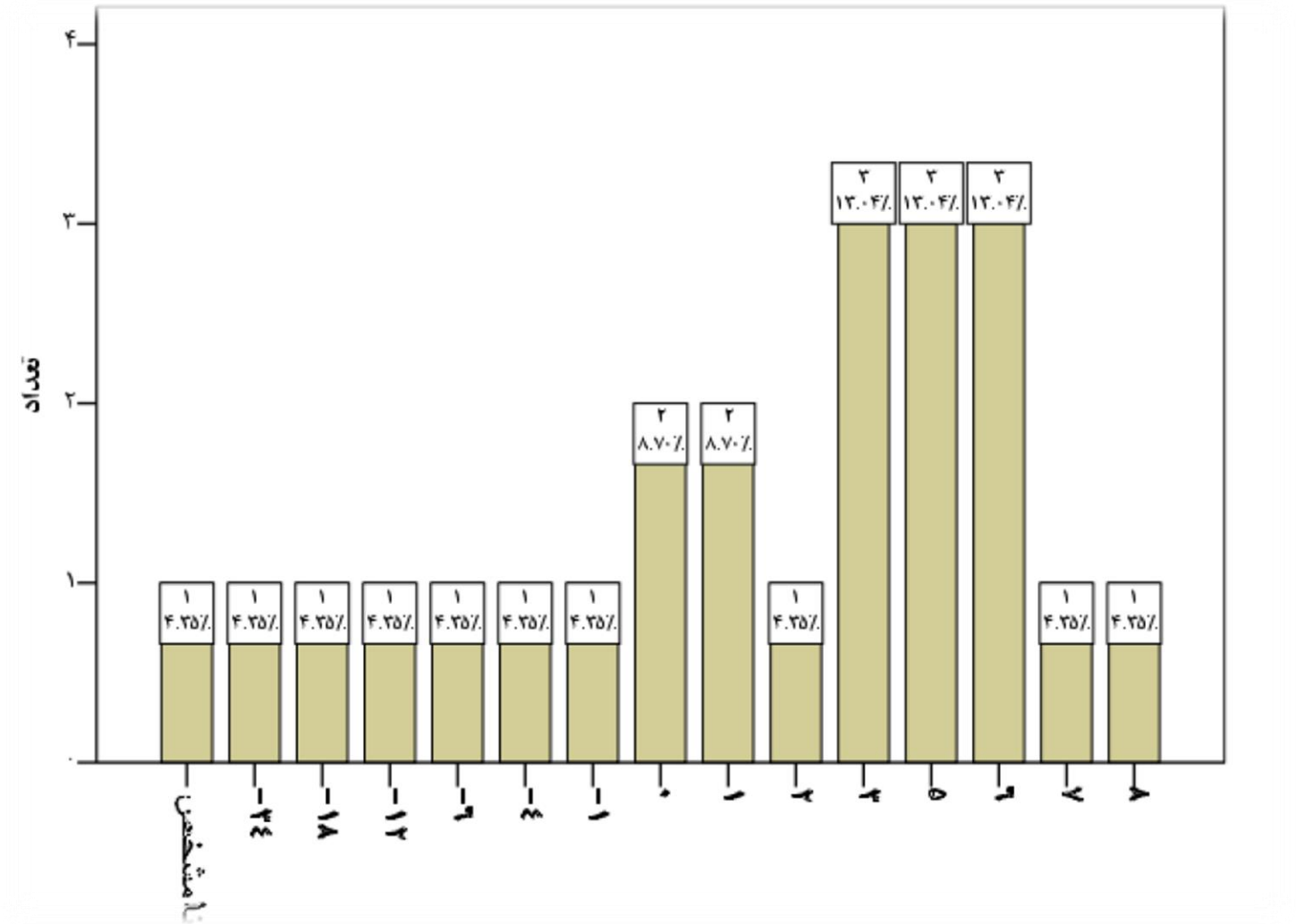
Clinical Features

Engraftment Rate



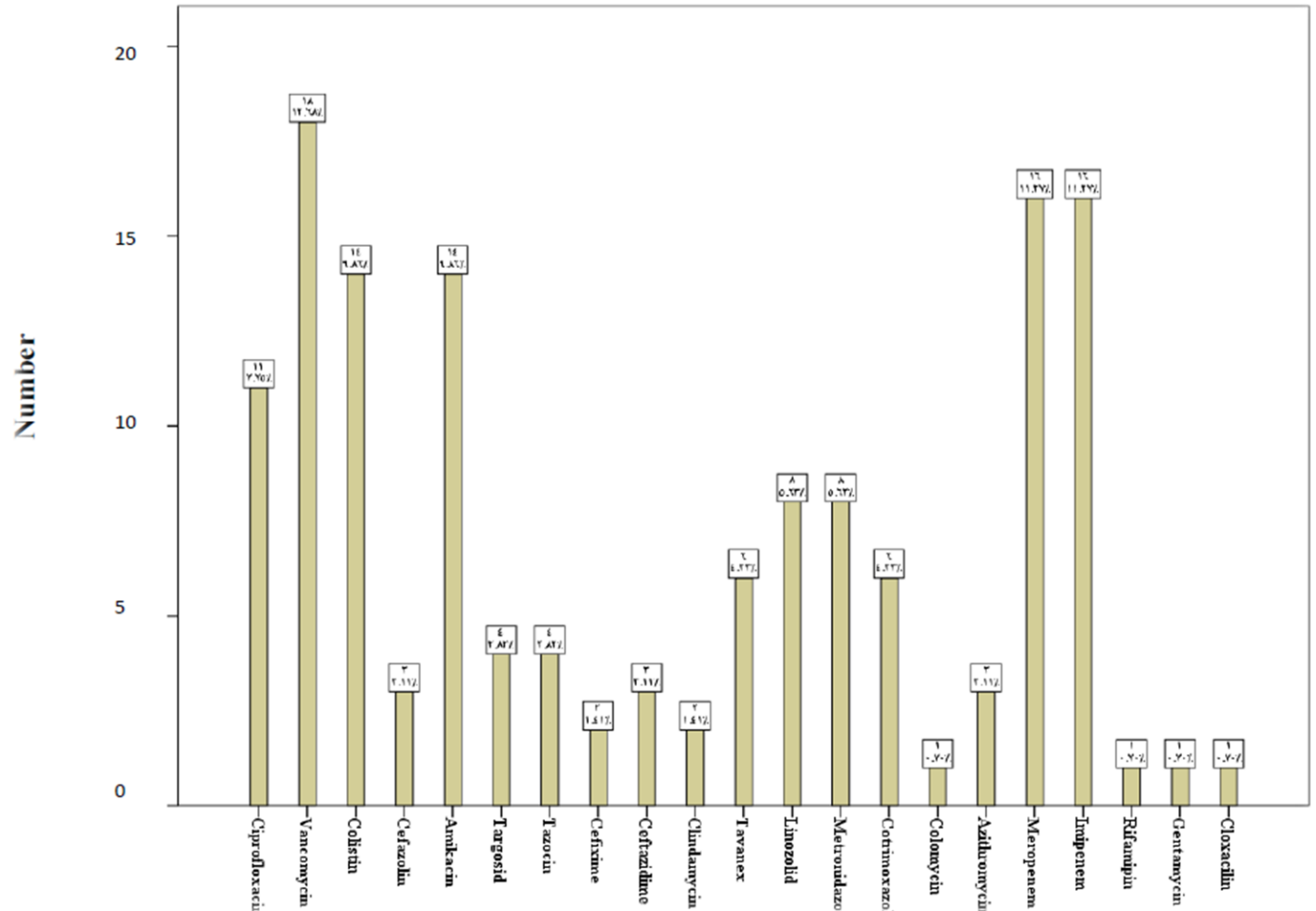
Clinical Features

Fiver starting day



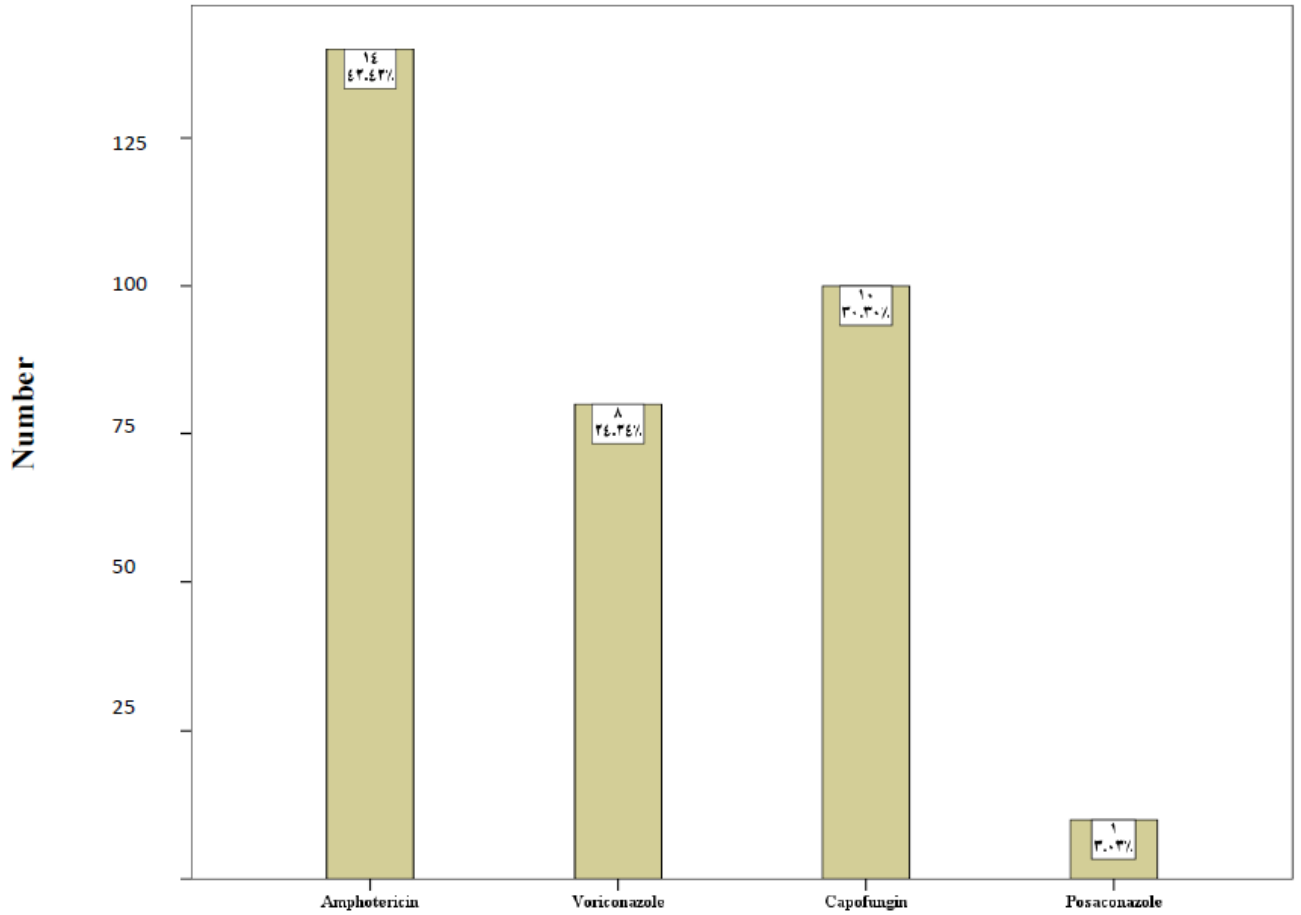
Clinical Features

Antibiotic drugs



Clinical Features

Antifungal drugs



## Clinical Features

Information related to age, number of fever days and granulocyte injection

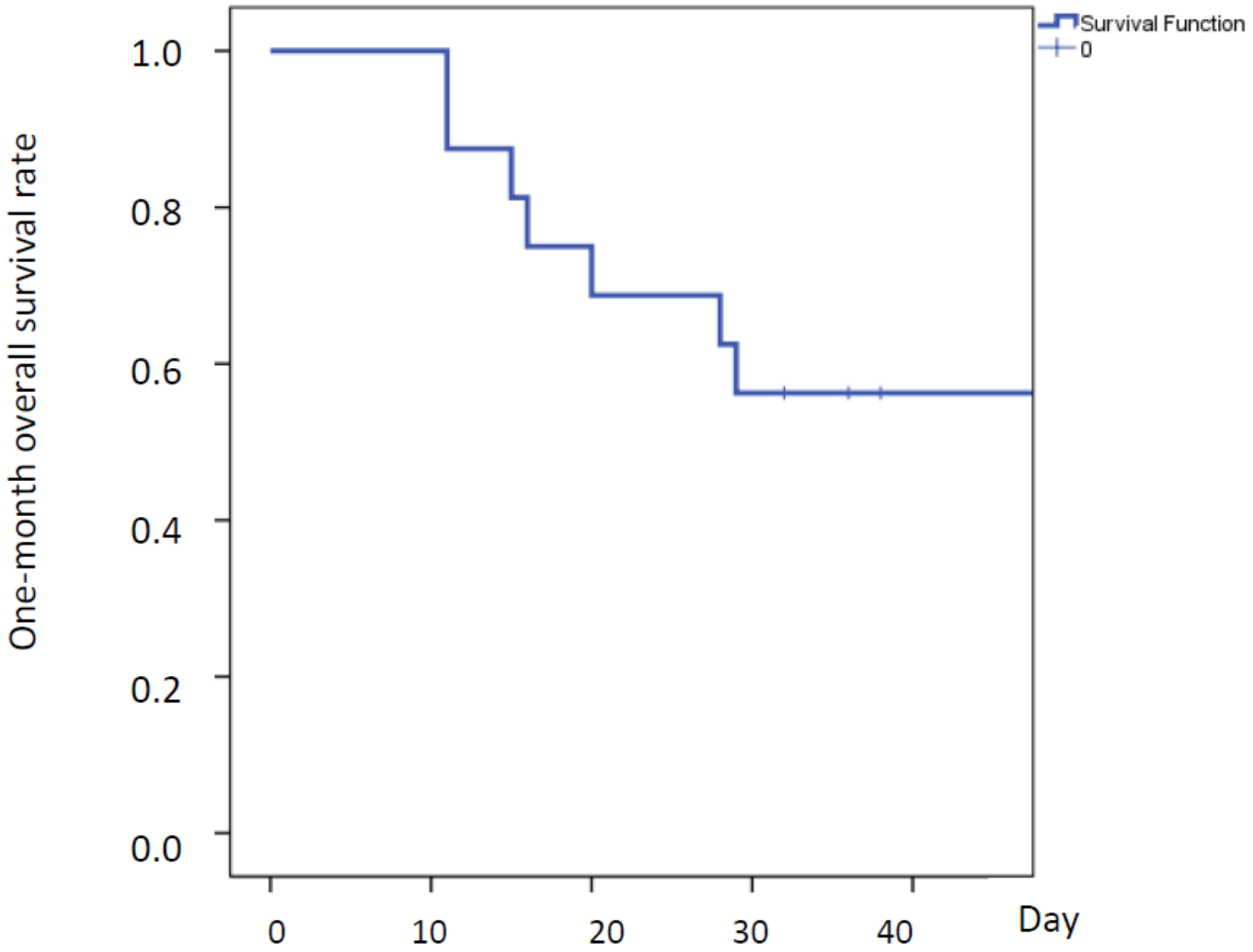
| Variable | Mean | Standard Division |
|---|---|---|
| Age | 38 | 13.27 |
| Number of injections | 1.62 | 1.20 |
| Number of days of fever before granulocyte injection | 11.43 | 12.17 |
| Number of days of fever after granulocyte injection | 9.19 | 9.03 |
| Number of antibiotics injected | 6.41 | 1.94 |
| WBC count after granulocyte injection | 528.57 | 628.09 |

## The interval between engraftment time and fever and granulocytes injection

| Variable | Mean | Standard deviation |
|---|---|---|
| Time of engraftement (day) | 13 | 4.56 |
| The interval between the onset of fever and granulocyte injection (days) | 11.43 | 17.64 |

## Overall Survival

## One-month overall Survival

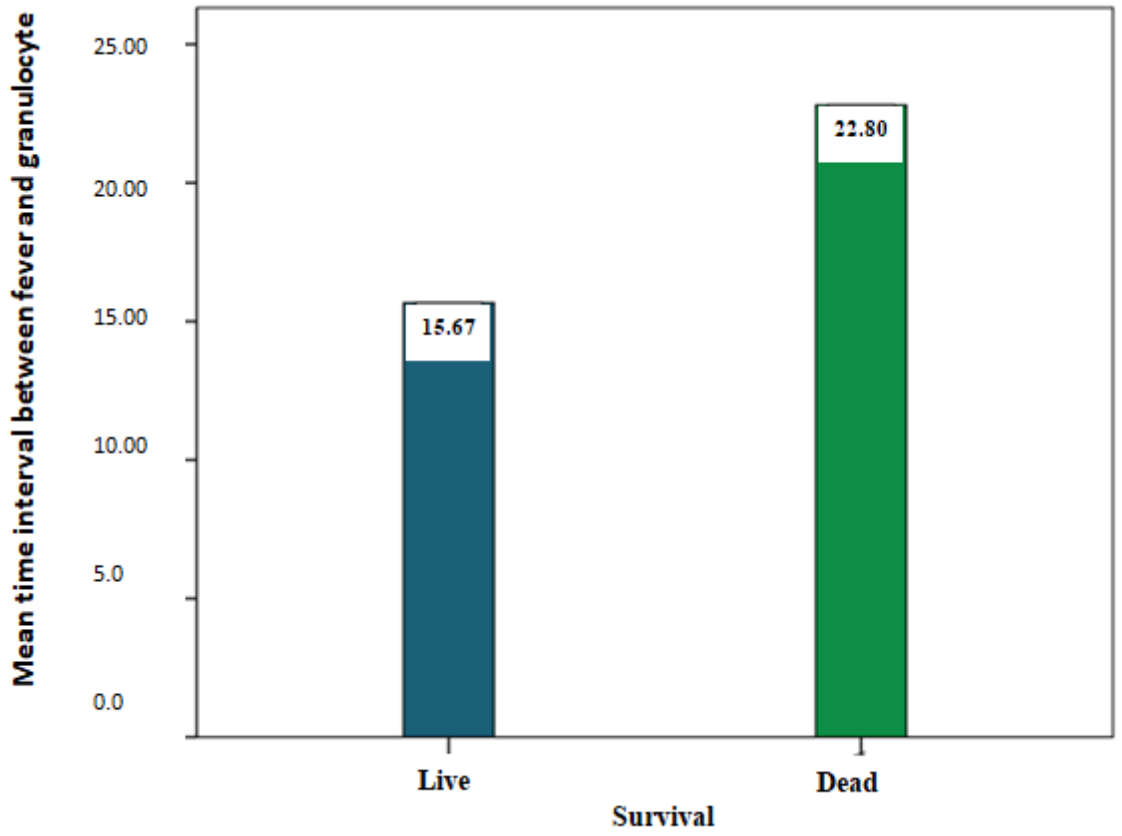



- **One month overall survival rate: 56%**

# survival

- moetality :12 patiens
- Aplastic anemia 5
- HD 3
- AML 2
- ALL 2
- 8 out of 12 died before engraftement

## The interval between engraftment time and fever and granulocytes injection




- According to the above graph, the average time interval between the onset of fever and the injection of granulocytes in the patients who died was longer than in the patients who survived.

# Review and discussion

# Granulocytes

- At least 1 × 1010 neu trophils would be required to achieve a therapeutic effect
- Whole blood-derived buffy coats contain 5 × 108 to 1 × 109 neutrophils
- advent of automated cell separators

# Donor Stimulation: Granulocyte colony stimulating factor (G-CSF) and Corticosteroids (CS)

- Mobilize grans. from BM into circulating pool
- Prolong PMN survival in vivo and in vitro
- G-CSF improves PMN quality
- CS: increase ANC **2-3-fold** over 4-24 hrs.
- G-CSF: increase ANC **7-10-fold**, peaking around

12 hrs, leading to > 1 x $10^{10}$ grans.

- G-CSF+CS: increases ANC **10-13.5-fold**
- GCSF adverse effects: h/a, bone and jt. pain, insomnia, CAD
- CS adverse effects: fluid accumulation, wt. gain, insomnia, posterior subcapsular cataracts

Drewniak 2009, Liles 2000, Ikemoto 2012, Stroncek 2001

# mobilization

- donors stimulated with steroids and containing approximately 1 to 2.5 × 1010 neutrophils
- Co-administration of G-CSF and a single dose of dexamethasone increases the yield to 4 to 8 × 1010 neutrophils or higher
- Identifying a suitable donor and administering medications prior to granulocyte apheresis requires at least 1 day of lead-time
- Many centers limit G-CSF-mobilized granulocyte collections to re-lated donors or friends of the patient
- The short-term side effects of G-CSF such as bone pain, headache, and myalgia are generally mild and treatable
- long-term follow-up of granulocyte donors stimulated with G-CSF and dexamethasone suggests that granulocyte donation is safe

# Harvesting agent: Hydroxyethyl Starch (HES)

- RBC-sedimenting agent; granulocytes at bottom of WBC layer (buffy coat) in centrifuge and similar in density to RBCs; hence such an agent creates better separation between the RBCs and grans.
- Added to donor blood as it enters centrifuge bowl of leukapheresis machine
- Adverse effects: fluid retention,

  mild wt. gain, allergic rxns., pruritus

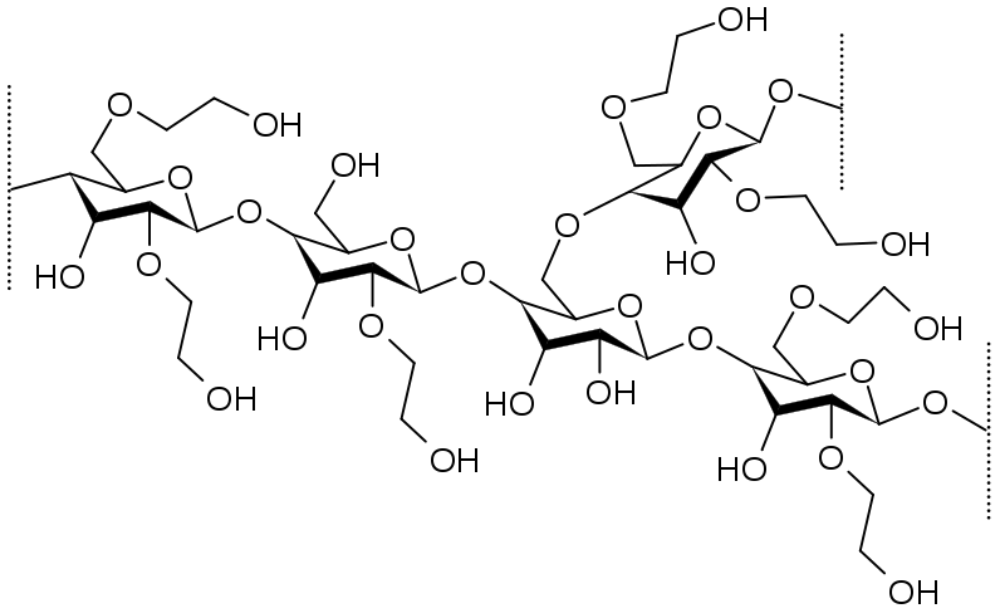


**Table 1**

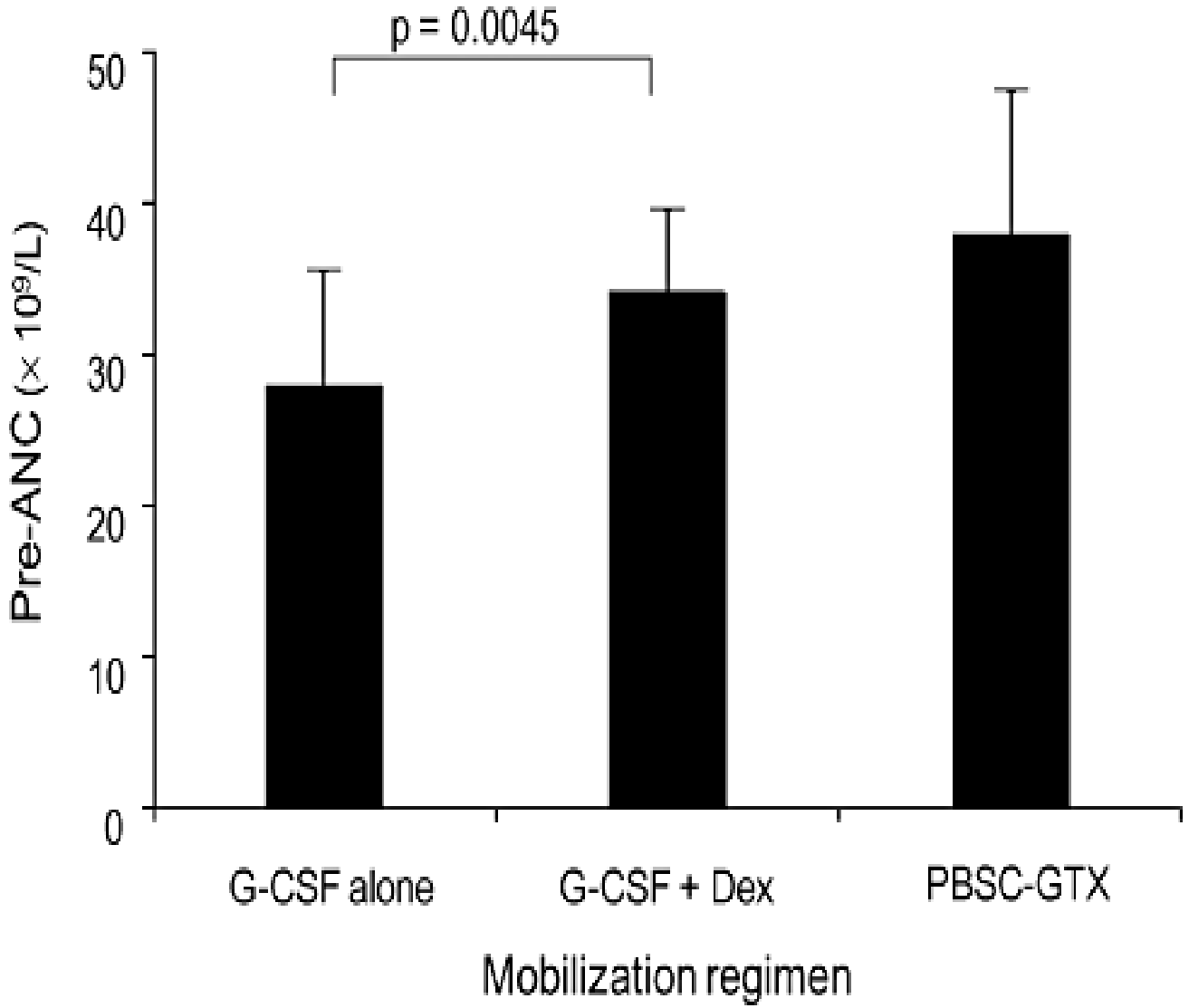
Content of granulocyte components according to procurement method.

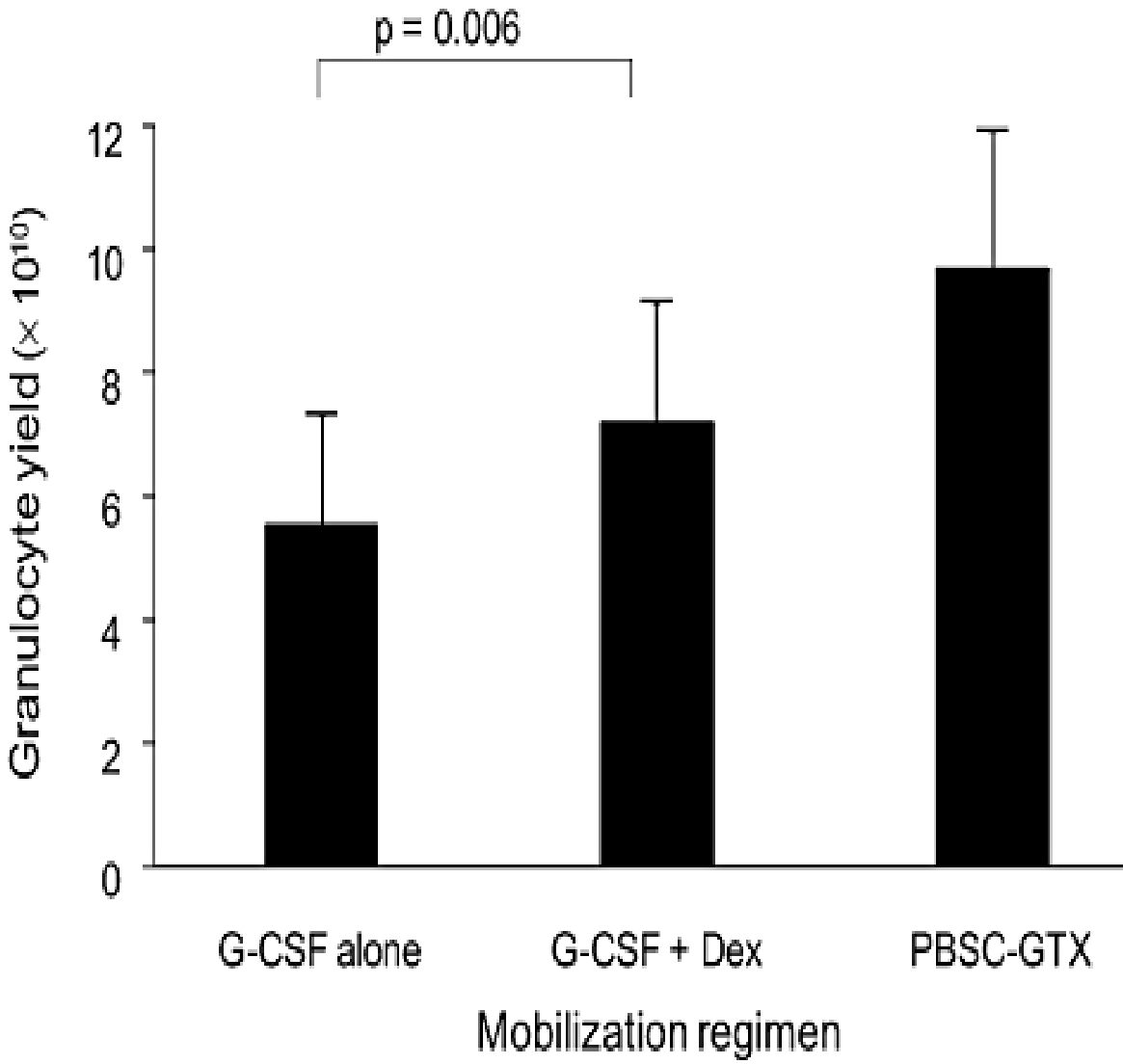
| | Volume (mL) | Hct (%) | Neutrophils ($\times 10^{10}$) | Lymphocytes ($\times 10^{9}$) | Platelets ($\times 10^{9}$) |
|---|---|---|---|---|---|
| Buffy Coat (single) [20] | 55-65 | 35-59 | 0.04-0.2 | 0.6-2.8 | 50-105 |
| Pooled Buffy Coat [20] | 232-272 | 18-24 | 0.7-1.17 | 5.2-8.2 | 226-585 |
| Apheresis (steroid only)[a] | 215-330 | 10-15 | 1.2-2.7 | 1.5-2.0 | 50-180 |
| Apheresis (G-CSF ± steroid)[a] | 300-375 | 10-15 | 4.0-8.4 | 1.5-5.0 | 50-250 |

[a] Internal data, NIH.

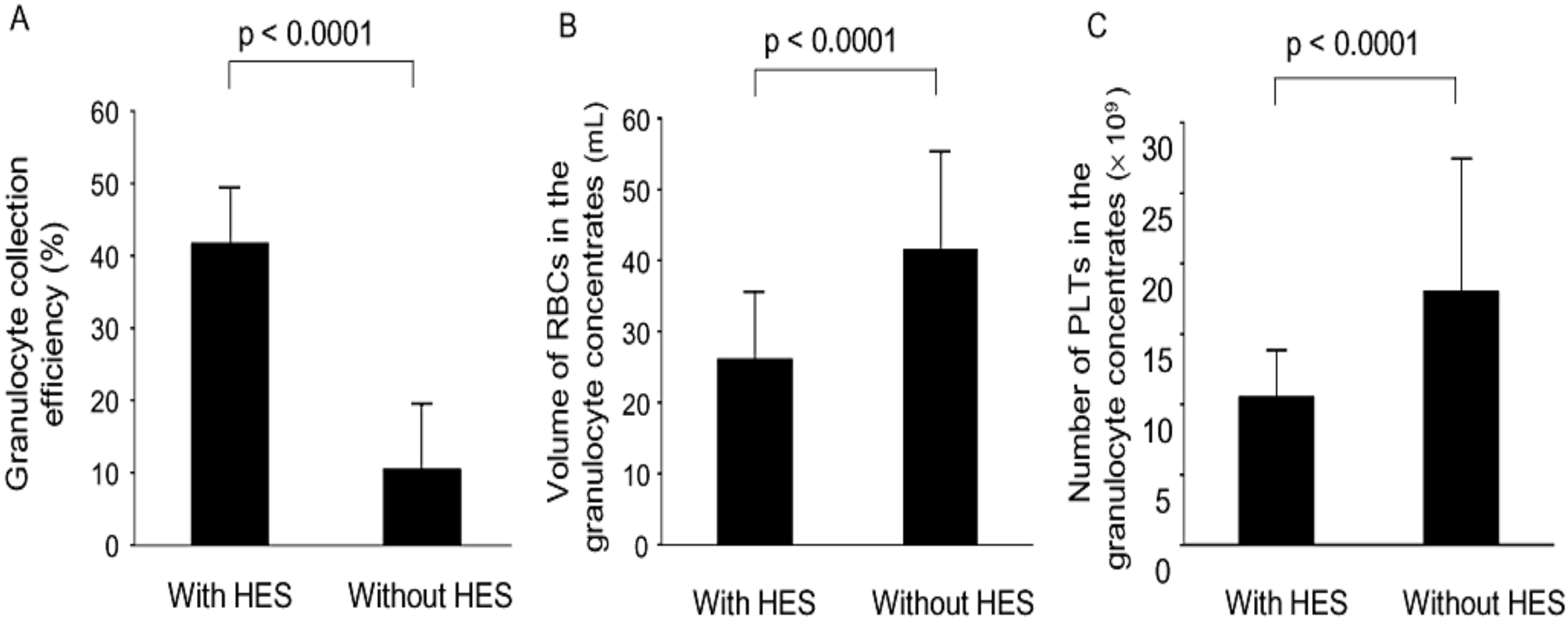
# Ikemoto et al. (2012)

- Retrospective study
- 600 mcg G-CSF + 8 mg dexa (n=68) vs. 600 mcg G-CSF alone (n=40) vs. peripheral blood stem cell (PBSC) donors (5 days of G-CSF, 400 mcg) (n = 15)
- Leukapheresis with HES (n = 108) and without HES (15)
- Post-mobilization ANC, gran. collection yield, and adverse effects measured



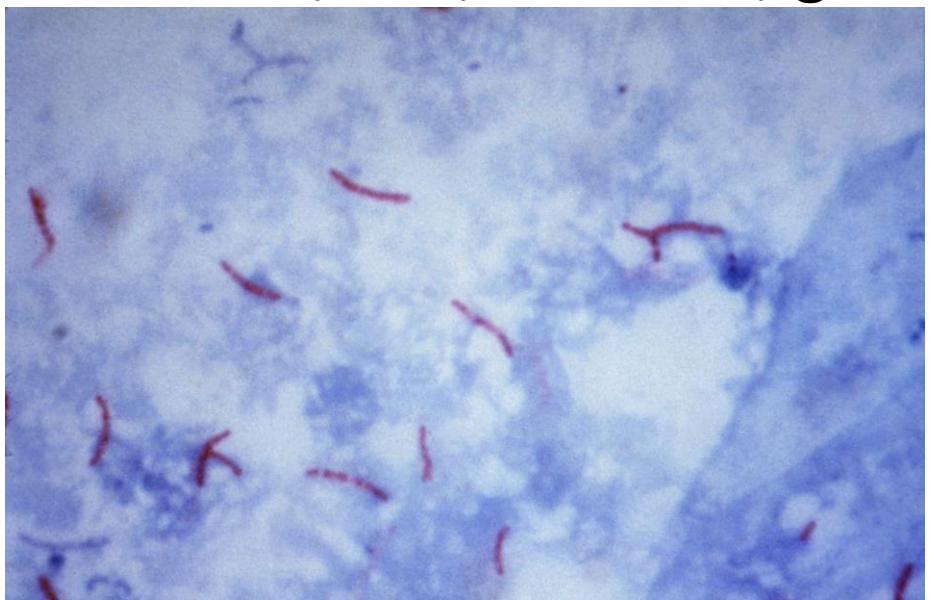

Ikemoto et al. 2012




Ikemoto et al. 2012

# Donor selection and qualifications

- HES does not remove all RBC, so should be ABO, Rh matched and crossmatch compatible
- HES Contraindications: allergies/hypersensitivity to starch; renal dysfunction, dialysis; intracranial bleed
- CS Contraindications: HTN, DM, GI ulcers, glaucoma, TB, fungal infections



# Donor selection and qualifications

- Community donors and directed donors, such as family and friends
- Screen with blood donor questionnaire
- Test for transfusion-transmitted infectious disease markers (IDMs)
  - HBV, HCV, HIV, syphilis, , HTLV, CMV,HSV,VZV,TOXO,EBV
- Results generally take 2-3 days; usually Pt. needs GTX before that!
- Institution may require the donor have a negative IDM within past 30 days; nevertheless, could have ID, so needs emergency release procedure and approval of BB director

# Donor Mobilization, Collection, and Processing

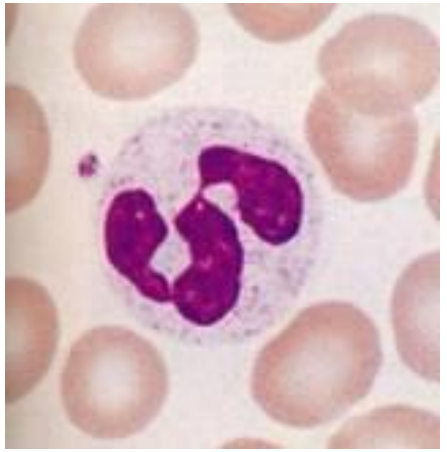
- Mobilization: G-CSF 200-600 mcg SC + dexamethasone 8 mg PO
- 8-16 hrs. prior to collection
- Leukapheresis (with HES)
- Duration: 3-5 hours, to process 7-10 L blood
- Label with unit #, blood type, collection date, expiration date, product volume, collection facility
- Irradiate with 25 grays (Gy): prevent GTX-associated GVHD
- Check WBC and diff. (gran. dose should be > 1 x 10e10; 1-2 x 10e9/kg for neonates);
  - Grans. Are not licensed by FDA but AABB standards requires at least 75% of products contain 1 x 10e10

# Practical points

- granulocytes are irra- diated to prevent transfusion-associated graft versus host disease
- stored at room temperature for a maximum of 24 hours to preserve adequate function
- the product should be cross match compatible with the recipient to prevent hemolytic transfusion reactions
- Transfuse STAT; begin slowly (e.g. 1-2 ml/hr); may premedicate with acetaminophen and/or diphenhydramine as the product is rich in cytokines and may cause fever and/or chills
- Granulocytes should be infused through a standard blood administration filter over 1 to 2 hours ,no leukocyte filter

# Clinical Indications

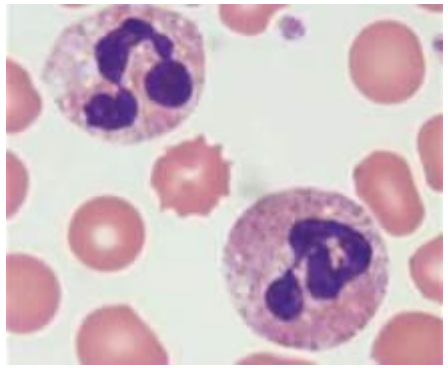
- No definitive indications
- Consensus opinion:
  - ANC < 500/mcL;
  - evidence of bacterial or fungal infection that's unresponsive to appropriate tx. (for > 48 hrs or life-threatening);
  - PMN recovery expected/curative tx. planned
- Chemotherapy and/or HSCT
- Aplastic anemia
- Neonatal sepsis
- Chronic granulomatous disease
- Prophylactic GTX



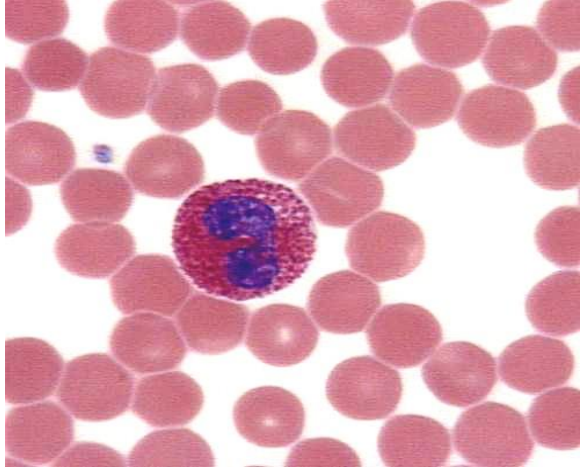
Elebute et al. 2006,
Marfin et al. 2013

# Contravesies

- newer antimicrobial drugs
- prophylactic  growth. factors
- A survey in England and North Wales reported little consensus among hematology units on the use of GTX
- The most commonly cited reason for reluctance to use GTX was lack of evidence of effect



# Practice guidelines



- In the USA
  - no FDA recognition of granulocyte concentrates as a licensed blood component
  - the Circular of Information (COI) outlines indications for which granu- locytes are typically used
- in France, Germany, and the UK
  - national guidance on the indications for therapeutic use of granulocytes is available
- In Canada, Australia and Brazil
  - there are standards or guidelines for collection and preparation but no official national recommendations on usage
-

# Outcomes: evidence of clinical efficacy

- The existing literature is predominantly
  - case reports
  - uncontrolled case series
- Cochrane database systematic review
  - low-grade evidence that prophylactic granulocyte transfusions in chemotherapy or HSCT decrease the risk of bacteremia or fungemia
  - primary prophylactic GTX is no longer recommended.

Case series of adult patients treated with GTX in the G-CSF era.

| Study | N | Study design | Response (Fungal) | Response (Bacterial) |
|---|---|---|---|---|
| Grigg (1996) [60] | 11 | Retrospective | 0% resolved | 100% resolved |
| Price (2000) [58] | 19 | Prospective | 0% with IA cleared infection | 100% resolution |
| Lee (2001) [39] | 25 | Prospective | 73% response | 45% response |
| Illerhaus (2002) [62] | 18 | Retrospective | 55% IPA responded | 78% septicaemia responded |
| Hubel (2002) [71] | 74 | Retrospective | 18% mould, 55% yeast stable | NS |
| Rutella (2003) [68] | 22 | Retrospective | 57% (0% response in IFI) | 54% response |
| Mousset (2005) [61] | 44 | Prospective | 78% response at 30 days | 92% response at 30 days |
| Safdar (2006) [104] | 20 | Retrospective | 45% CR or PR, 15% stable | NS |
| Ofran (2007) [105] | 47 | Retrospective | 64% infection-related survival | 53% infection-related survival |
| Quillen (2009) [66] | 32 | Retrospective | 44% survival | 58% overall survival to discharge |
| Al-Tanbal (2010) [106] | 22 | Retrospective | 75% survival | 68% clinical improvement |
| Ang (2011) [107] | 15 | Retrospective | 31% cleared | 63% cleared |
| Kim (2011) [91] | 128 | Retrospective | 47% control of IFI | 53% overall control of infection |
| Safdar (2014) [108] | 74 | Retrospective | 45% patients had IFI | 46% overall response |
| Wang (2014) [109] | 56 | Retrospective | 87% 30-day survival | 92% 30-day survival |
| Marciano (2017) [88] | 40 | Retrospective | 82% success | 94% success |

CR = complete response; IFI = invasive fungal infection; IA = invasive aspergillosis; IPA = invasive pulmonary aspergillosis; PR = partial response; NS = not specified.

**Table 3**

Case series including pediatric patients treated with GTX in the G-CSF era.

| Study | N | Study design | Response (Fungal) | Response (Bacterial) |
|---|---|---|---|---|
| Grigull (2006) [84] | 32 | Retrospective | 67% survival | 81% survival |
| Kikuta (2006) [80] | 13 | Prospective | 50% response | 73% response |
| Sachs (2006) [81] | 27 | Prospective | 100% response | NS |
| Drewniak (2008) [76] | 18 | Prospective | 73% response | 100% response |
| Seidel (2009) [82] | 69 | Prospective | 28-day survival probability $0.51 \pm 0.12$ | 28-day survival probability $0.89 \pm 0.06$ |
| Graham (2009) [83] | 13 | Retrospective | 50% survived to discharge | 100% survived to discharge |
| Atay (2011) [78] | 35 | Retrospective | 55% clinical response | 65% clinical response |
| Ozturkmen (2013) [77] | 10 | Retrospective | 50% response | 80% response |
| Diaz (2014) [79] | 18 | Retrospective | 80% response | 100% response |
| Nikolajeva (2015) [110] | 28 | Retrospective | 79% 100-day survival | 50% 100-day survival |

Practice considerations.

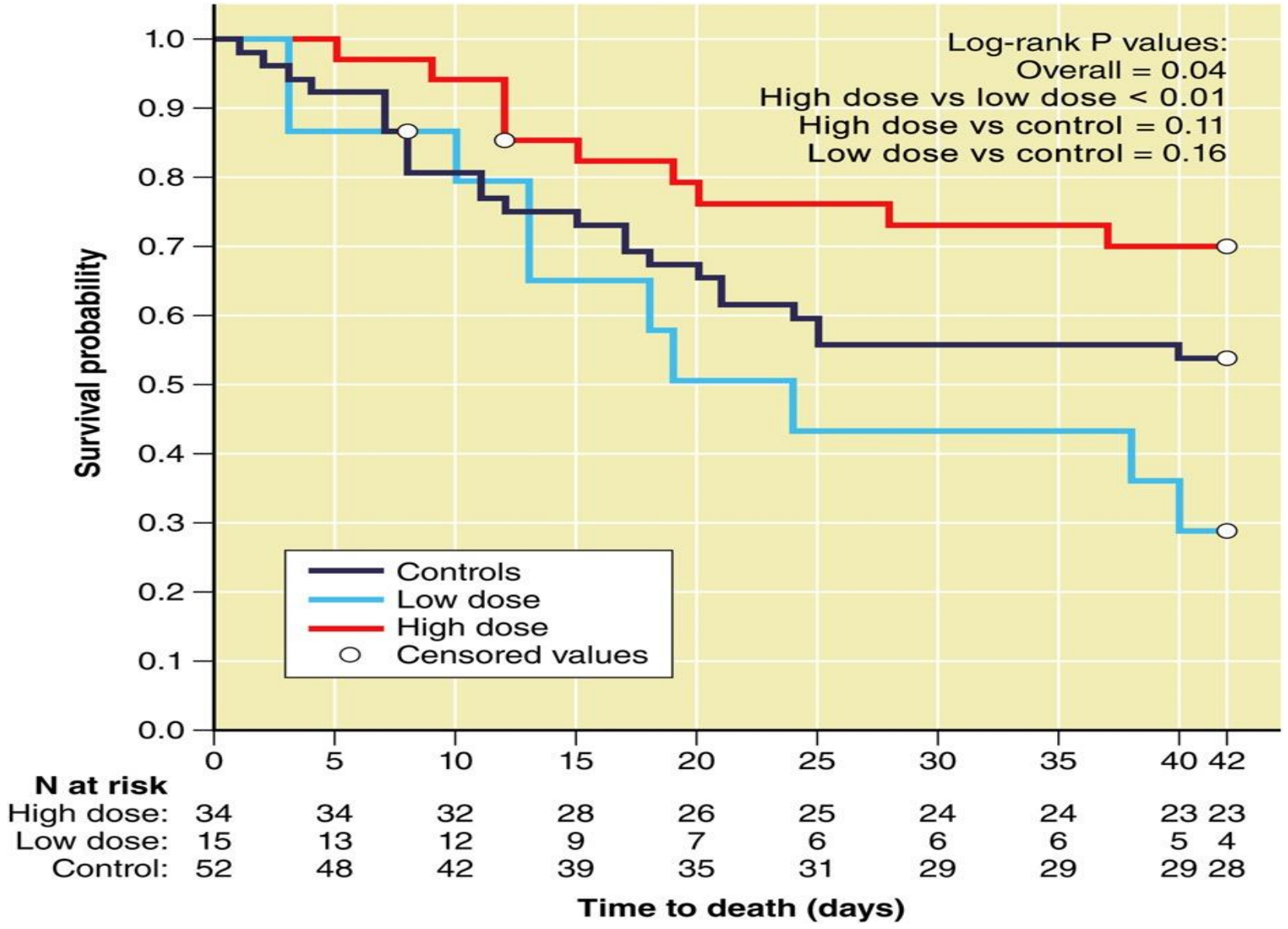
When considering a course of GTX for a patient, determine:

Is GTX **indicated**?

# RING TRIAL the largest randomized controlled trial of granulocyte transfusions

- 114 patients with neutrophil count <0.5 × 109 /L and proven or probable bacterial or fungal in- fection
- The primary endpoint :survival plus microbial response at 42 days.
- Differences in primary endpoint success rates for granulocyte and control arms were not statistically significantly different for any infection type.
- The granulocyte dose was also lower than anticipated; the target of ≥4.0 × 1010 neutrophils per transfusion was only achieved in 70% of subjects.
- In a post-hoc analysis, subjects who received an average dose per transfusion of ≥ 0.6 × 109 granulocytes/kg tended to have better outcomes than those receiving a lower dose.

# Ring study


Log-rank P values:
Overall = 0.04
High dose vs low dose < 0.01
High dose vs control = 0.11
Low dose vs control = 0.16
Survival probability
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Controls
Low dose
High dose
Censored values
0 5 10 15 20 25 30 35 40 42
N at risk
High dose: 34 34 32 28 26 25 24 24 23 23
Low dose: 15 13 12 9 7 6 6 6 5 4
Control: 52 48 42 39 35 31 29 29 29 28
Time to death (days)

# Outcomes: evidence of clinical efficacy

- A sys tematic Cochrane review of therapeutic granulocyte transfusion
  - There were no clear differences in other outcomes
  - insufficient data to report on risks of harm
- it was not possible to establish whether granulocyte transfusions affect all-cause mortality.

# IN PEDIATRICS

- . small size, so may obtain a superior increment in ANC post-GTX compared to adults
- however one prospective study showed that neither body weight nor granulocyte dose impacted infection outcome and sur- vival in pediatric patients
- More modest responses were re- ported in other series, with promising short-term survival but the majority of patients ultimately dying of their infection
- Some studies suggest a role for granulocyte transfusions in preventing infections or progression of infections in children with anticipated prolonged neutropenia after HSCT or chemotherapy

# CGD/NEONATES

- Case reports and small series of children and young adults with CGD report benefit from the addition of GTX to the therapeutic arsenal . The largest cohort of CGD patients with refractory infections treated with of GTX as an adjunctive therapy reported that overall >80% infections improved with this approach .
- To date, no randomized controlled trials of GTX have been con-ducted in children.
- A Cochrane review concluded that there is in- conclusive evidence from randomized controlled trials (RCTs) to support or refute the routine use of granulocyte transfusions in neutropenic, septic neonates

# adverse effects of granulocyte transfusion

- Febrile and allergic reactions 10%-15%
- symptomatic management with antipyretics will usually suffice.
- CMV . Since granulocyte recipients often require CMV-safe blood products, CMV seronegative donors are generally recommended for seronegative recipients.
- Pulmonary complications, including hypoxemia, hemoptysis, pulmonary infiltrates, acute lung injury and fluid overload reported in 10%-18%

# adverse effects of granulocyte transfusion

- amphotericin B during GTX  increase de- velopement of respiratory deterioration, keep some interval between
- alloimmunization to HLA and human neutrophil antigens , resulting in subsequent platelet and leukocyte transfusion refractoriness, or rejection of a subsequent allogeneic HSCT
- the RING study group reported no statistically significant difference in WBC alloimmunization in the GTX arm, and no demonstrable effect of the presence of alloimmunization on survival and micro- bial response at 42 days, the occurrence of transfusion reactions, or neutrophil increments

# future considerations

- in the future, granulocyte concentrates may be produced in advance, cryopre- served and made available as an off-the-shelf product.