EHA-GBMTA-AHA Hematology Tutorial: New aspects in diagnostic choices and treatment options of hematological malignancies

Session: Immunotherapy in Myeloid diseases

Self-assessment case 1

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Case 1

A 32 years old man came to emergency unit due to fever and moderate dyspnea. His medical history was unremarkable except for an episode of cold a couple of weeks ago. On exam, his temperature was 39℃ and pulse rate 100/min. He was alert with a GCS score 15.

Q1) Which one of these tests is NOT immediately needed for this patient?

1. Full blood count, including reticulocyte count and blood film review
2. Chest Xray
3. Blood Culture
4. Next generation sequencing for constitutional gene mutations
5. Viral Screening including HIV

Q1) Which one of these tests is NOT immediately needed for this patient?

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2. Chest Xray
3. Blood Culture
4. Next generation sequencing for constitutional gene mutations
5. Viral Screening including HIV

Case 1: Diagnosis

In patient with such acute presentations, it is important to exclude/ manage acute and life-threatening causes. Therefore, NGS is not a priority in this patient.

Results

- WBC 1.5 x 10^9/L
- Hemoglobin 69 g/L
- Neutrophil count 0.4 x 10^9/L
- Platelet count <40 x 10^9/L
- Reticulocyte count 50 x 10^9/L
- Chest x-ray is clear
- Virus screen negative
- No poorly differentiated cells or red cell fragments in the peripheral blood smear.
- Renal and liver profiles as well as LDH were normal.
- No other significant findings in clinical exam.

Q2) What is the best next step?

1. Bone marrow aspiration and biopsy (BMB)
2. Flow cytometry panel for B cell Lymphoma.
3. T cell subsets.
4. Immunoglobulin subclasses.

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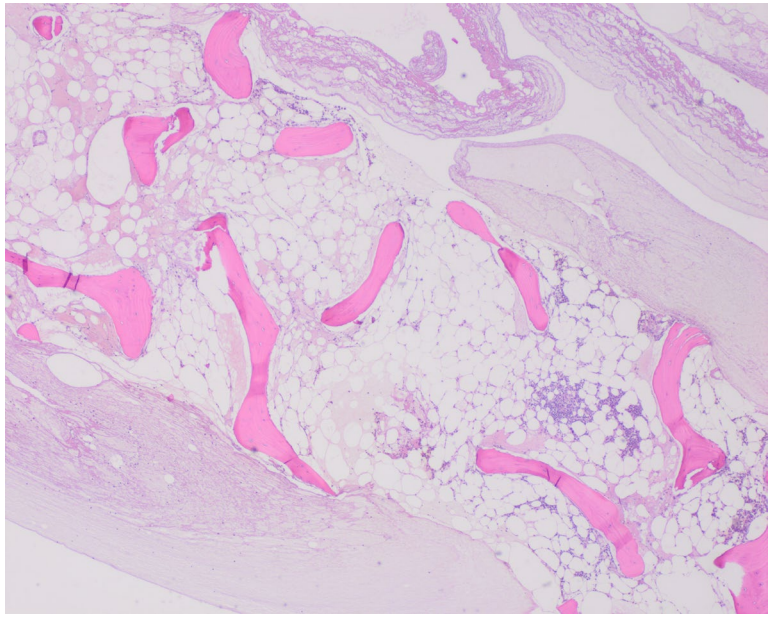
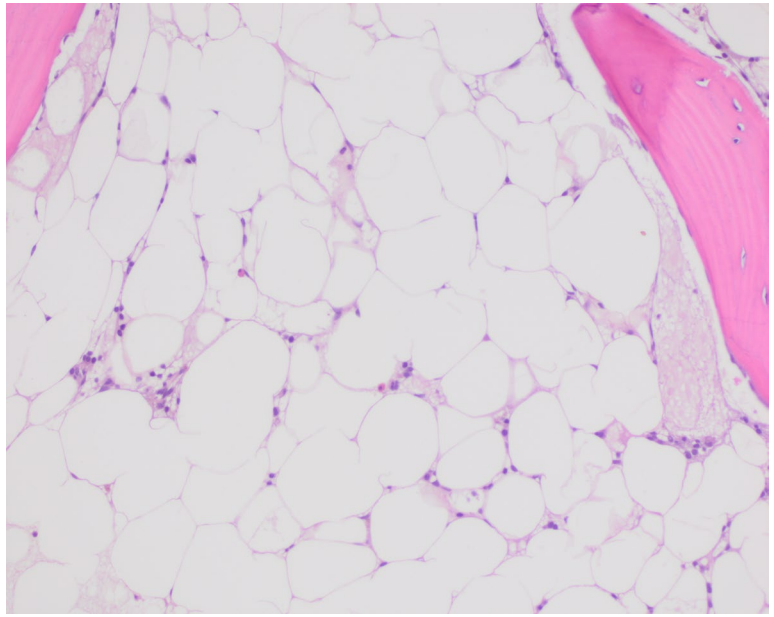
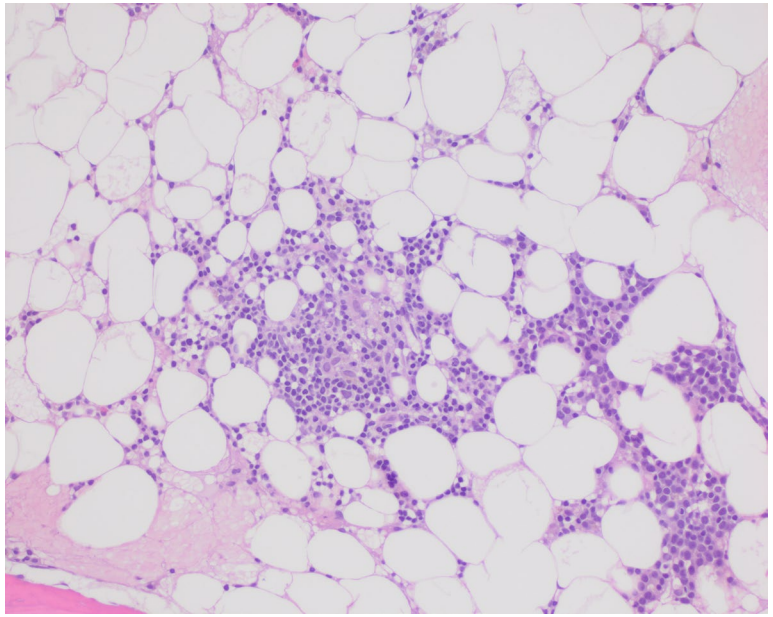
Case 1: Diagnosis

Considering very low blood counts and no clear evidence of other causes of acute cytopenia, bone marrow failure is one of the main differential diagnosis. Therefore, a BMB is the logical next diagnostic step.

Bone Marrow Biopsy

- Bone marrow biopsy shows a very hypocellular marrow (~10% cellularity).
- No malignant cells seen.
- No fibrosis (WHO grade 0).

Courtesy of Dr Sanam Loghavi, MD Anderson, Tx, USA

Q3) Which one of these investigations do you recommend next?

1. The ADAMTS13 test
2. Flow cytometry panel for malignant cells.
3. Flow cytometry for Paroxysmal Nocturnal Hemoglobinuria (PNH) clone and Myeloid Gene Mutations Panel.
4. Immunoglobulin subclasses.

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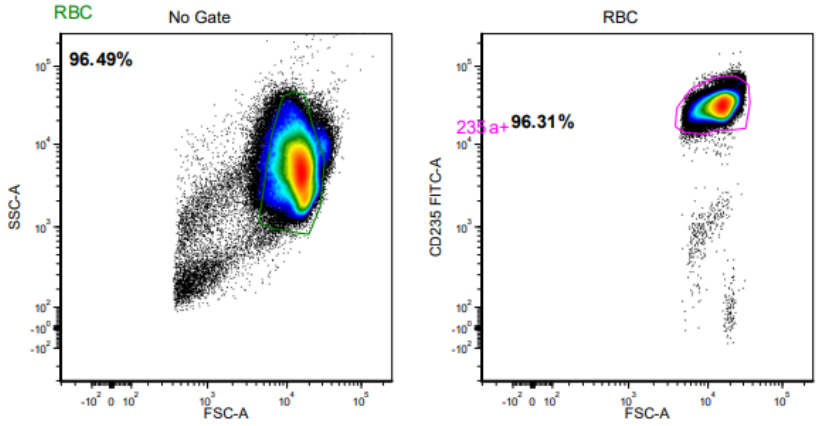
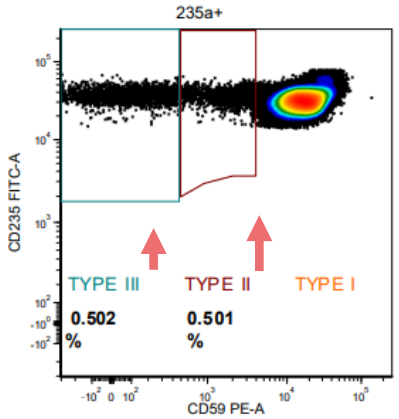
Case 1: Diagnosis

It is important to rule out all causes of BMF in this patient. His BMB is extremely hypocellular with no evidence for malignancy. Within the suggested tests, considering the lack of evidence for TTP, acute malignancies or history of immunodeficiencies, flow cytometry for PNH clone and Myeloid Gene panel are the most useful tests to request at this stage.

BMF, Bone marrow failure

Flow cytometry for Paroxysmal Nocturnal Hemoglobinuria (PNH) clone

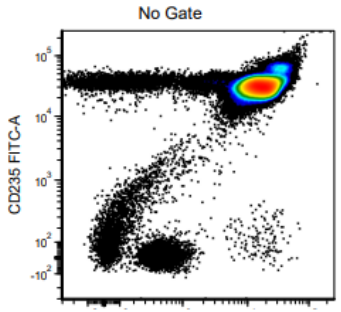


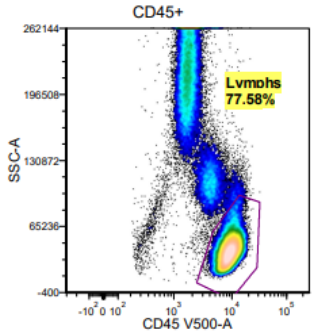


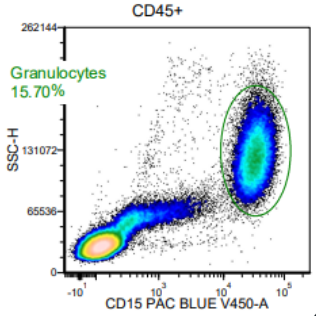
Total RBCs = 486240
Total PNH clone = 1.003

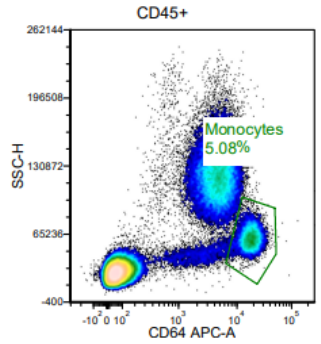
Type II Events = 2438
Type III Events= 2440

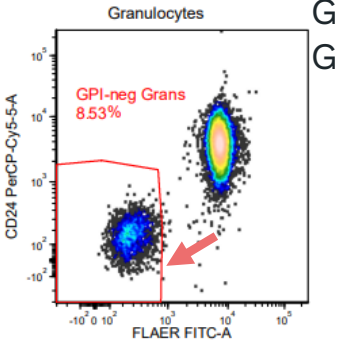
Type II clone size = 0.501 %
Type III clone size = 0.502 %

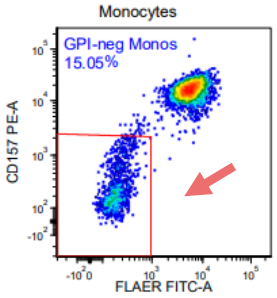








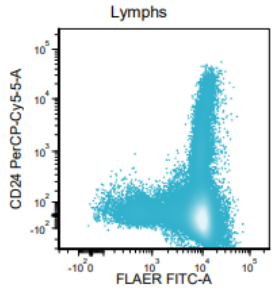




Total Events=159643
CD45 cells=151665
Granulocytes=23808
Monocytes=7699

GPI-neg Grans=2030
GPI-neg Monos=1159

Clone sizes:
GPI-neg Grans = 8.53%
GPI-neg Monos = 15.05 %



GPI⁻ Granulocytes= 8.5%
GPI⁻ Monocytes= 15.05%
GPI⁻ RBCs= 1.003

Courtesy of Dr Sanam Loghavi, MD Anderson, Tx, USA

Next Generation Sequencing (NGS) for Myeloid Gene Mutations (81 genes)

- Diploid karyotype
- No mutations on NGS (81 gene myeloid panel)

Courtesy of Dr Sanam Loghavi, MD Anderson, Tx, USA

Q4) What is the most likely diagnosis?

1. Acute Myeloid Leukemia (AML)
2. Myeloproliferative Neoplasia (MPN)
3. Myelodysplastic Neoplasm (MDS)
4. Immune thrombocytopenia (TP)
5. Aplastic Anemia (AA)

Q4) What is the most likely diagnosis?

1. Acute Myeloid Leukemia (AML)
2. Myeloproliferative Neoplasia (MPN)
3. Myelodysplastic Neoplasm (MDS)
4. Immune thrombocytopenia (TP)
5. Aplastic Anemia (AA)

Case 1: Diagnosis

A very hypoplastic marrow, pancytopenia, presence of PNH clone, no myeloid gene mutation and lack of malignant cell infiltration or fibrosis in the bone marrow, immune mediated aplastic anemia (AA) is the most likely diagnosis.

Q5) What treatment do you recommend in this patient?

1. Prednisolone
2. Anti thymocyte globulin (ATG) and Cyclosporine (Ciclosporin)
   +/- Eltrombopag
3. Danazol
4. Ruxolitinib
5. Watch and wait plus supportive care

Q5) What treatment do you recommend in this patient?

1. Prednisolone
2. Anti thymocyte globulin (ATG) and Cyclosporine (Ciclosporin) +/- Eltrombopag
3. Danazol
4. Ruxolitinib
5. Watch and wait plus supportive care.

Case 1: Treatment

As discussed during the lecture, immunosuppressive therapy is the standard of care in Severe AA. The addition of eltrombopag is becoming routine as first line in many (but not all) centers.

Outcome

- Partial response to ATG+ Cyclosporine.
- Still waiting for a matched donor for HSCT.

Q6) What treatment do you recommend next?

1. Prednisolone
2. Danazol
3. Ruxolitinib
4. Cyclosporine + Eltrombopag
5. Immune cell therapy with regulatory T cells (Tregs)

Q6) What treatment do you recommend next?

1. Prednisolone
2. Danazol
3. Ruxolitinib
4. Cyclosporine + Eltrombopag.
5. Immune cell therapy with regulatory T cells (Tregs).

Case 1: Treatment

The addition of eltrombopag to standard immunosuppressive therapy (IST) improved the rate, rapidity, and strength of hematologic response among previously untreated patients with severe aplastic anemia[1].
It can be considered in patients who did not respond to Standard IST.

[1]Peffault de Latour R, Kulasekararaj A, Iacobelli S, Terwel SR, Cook R, Griffin M, et al. Eltrombopag Added to Immunosuppression in Severe Aplastic Anemia. New England Journal of Medicine. 2022 Jan 6;386(1):11–23.

Next steps

No complete response following treatment with eltrombopag and still transfusion dependent .

Clinical Trials

Immune cell therapy with Tregs for immune-mediated BMF is under investigation. Although their efficacy has yet to be confirmed, the preliminary data suggest these treatments are safe and the results have been encouraging so far.

- **Autologous Tregs for Aplastic Anaemia (TIARA),** ClinicalTrials.gov ID NCT05386264 Sponsor King's College Hospital NHS Trust (Open).
- **A Clinical Trial of CK0801 (a New Drug) in Patients With Bone Marrow Failure Syndrome (BMF),** ClinicalTrials.gov ID NCT03773393, Sponsor Cellenkos, Inc. (Active, not recruiting).

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Self-assessment case 2

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Case 2

Patient was a 39 years old woman (in 2005).

She was diagnosed with severe AA while pregnant in 2005. ATG (horse) started after delivering her child, followed by rabbit ATG which led to complete response.

She had a PNH clone from 2005 to January 2019. PNH clone undetectable in March 2019.
Myeloid Gene Mutation Panel was not routinely available at the time of her initial bone marrow biospy.

Since 2018, a rise in Hb was noted accompanied by migraine, itching, rash and fatigue.

Case 2; Investigations

- WBC 12.94 x 10^9/L
- Hemoglobin 161 g /L
- Hct 0.48
- Lymphocytes 0.99 x 10^9/L
- Neutrophil count 5.09 x 10^9/L
- Platelet count 556 x 10^9/L
- Eosinophil Count 3.9 x 10^9/L
- Basophil Count 0.25 x 10^9/L
- Monocyte Count 2.66 x 10^9/L

Additional investigations (2020):

- *JAK2*, *CALR*, *cMPL* were wild type.
- No evidence of a *BCR::ABL1* rearrangement.
- Erythropoietin 2.7 IU/L (5-25).
- FIP1L1-PDGFRA negative.

Q1) What is the best next step?

1. Venesection to keep Hct below 0.45
2. Start Hydroxycarbamide with the diagnosis of triple negative Polycythemia Vera (PV)
3. Start Pegylated Interferon with the diagnosis of Essential Thrombocythemia (ET)
4. Bone Marrow biopsy plus Myeloid gene mutation panel and RNA fusion panel
5. Watch and wait

Q1) What is the best next step?

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4. Bone Marrow biopsy plus Myeloid gene mutation panel and RNA fusion panel.
5. Watch and wait

Case 2:Diagnosis

Considering the patient's past history, abnormally high Hb and Platelet count as well as high eosinophile, monocytes and basophile, her bone marrow needs to be reviewed for potential transformation and accurate diagnosis before any treatment, which could affect the BM features.

Q2) What is the best next step while waiting for the results?

1. Venesection to keep Hct below 0.45 and control symptoms.
2. Start Hydroxycarbamide with the diagnosis of triple negative Polycythemia Vera (PV).
3. Start Pegylated Interferon with the diagnosis of Essential Thrombocythemia (ET).
4. Watch and wait.

Q2) What is the best next step while waiting for the results?

1. Venesection to keep Hct below 0.45 and control symptoms.
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3. Start Pegylated Interferon with the diagnosis of Essential Thrombocythemia (ET).
4. Watch and wait.

Case 2: Management

As patient is symptomatic, venesection is the safest approach at this stage.

Case 2: Diagnosis

- A bone marrow trephine (2020) was non diagnostic but focal clusters of megakaryocytes and a mild increase in reticulin reported. Monocytosis noted and MDS/MPN could not be excluded, although no overt dysplasia.
- Fusion panel was negative.
- Her symptoms improved slightly with venesection.
- Myeloid Gene Mutations Panel: ~~The~~ ASXL1 and SETBP1 mutations were detected in 2020.
- As there was no clear indication of MDS or MPN in BMB and symptoms were controlled, patient followed with watch and wait.
- However, in 2022, patient became symptomatic again and needed venesection.

Blood counts in 2022

- WBC 8.4 x 10^9/L
- Hemoglobin 133 g/L
- Hct 0.419 (after venesection)
- Lymphocytes 0.9 x 10^9/L
- Neutrophil count 5.09 x 10^9/L
- Platelet count 570 x 10^9/L
- Eosinophil Count 0.7 x 10^9/L
- Basophil Count 0.8 x 10^9/L
- Monocytes 1.8 x 10^9/L

Q3) What do you suggest as the next step?

1. Only continue venesection to keep Hct below 0.45.
2. Start Hydroxycarbamide with the diagnosis of triple negative Polycythemia Vera (PV).
3. Repeat Myeloid Gene Mutation Panel and BMB
4. Watch and wait.

Q3) What do you suggest as the next step?

1. Only continue venesection to keep Hct below 0.45.
2. Start Hydroxycarbamide with the diagnosis of triple negative Polycythemia Vera (PV).
3. Repeat Myeloid Gene Mutation Panel and BMB
4. Watch and wait.

Case 2: Management

By 2023 the patient is symptomatic, and venesection is needed more often. We would like to know whether any of the MPN related mutations or new mutations are detectable at this stage?

Case 2

- Myeloid Gene Mutations Panel 2023: The same ASXL1 and SETBP1 mutations were detected as in 2020 and **additional STAT5B mutation noted ~~(2023).~~**
- Considering the patient remained symptomatic, finding the new mutation, and no definite diagnosis, BMB repeated in 2024.

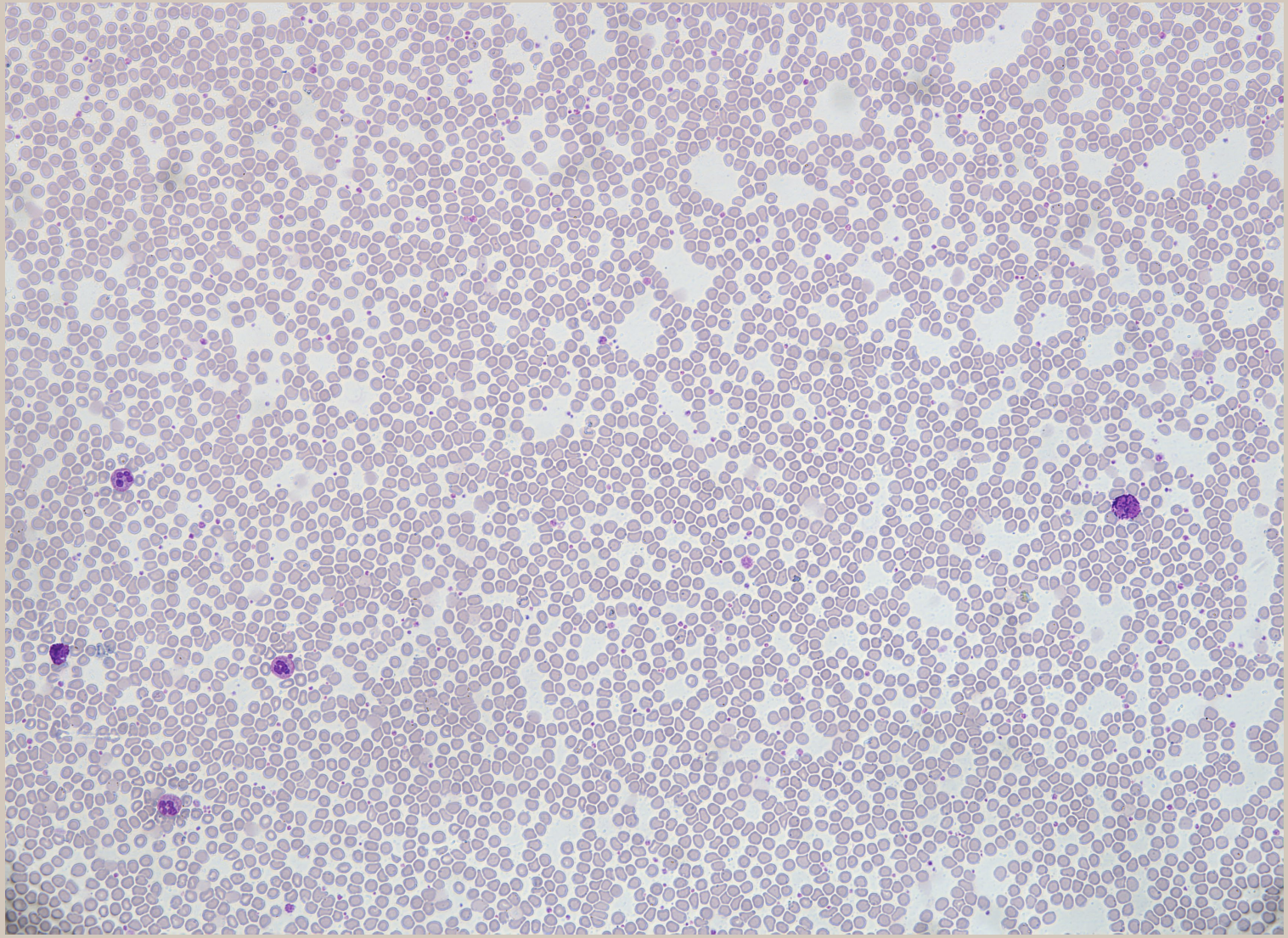
Peripheral blood x 20

No leucocytosis

Hb 134g/L hct 0.43

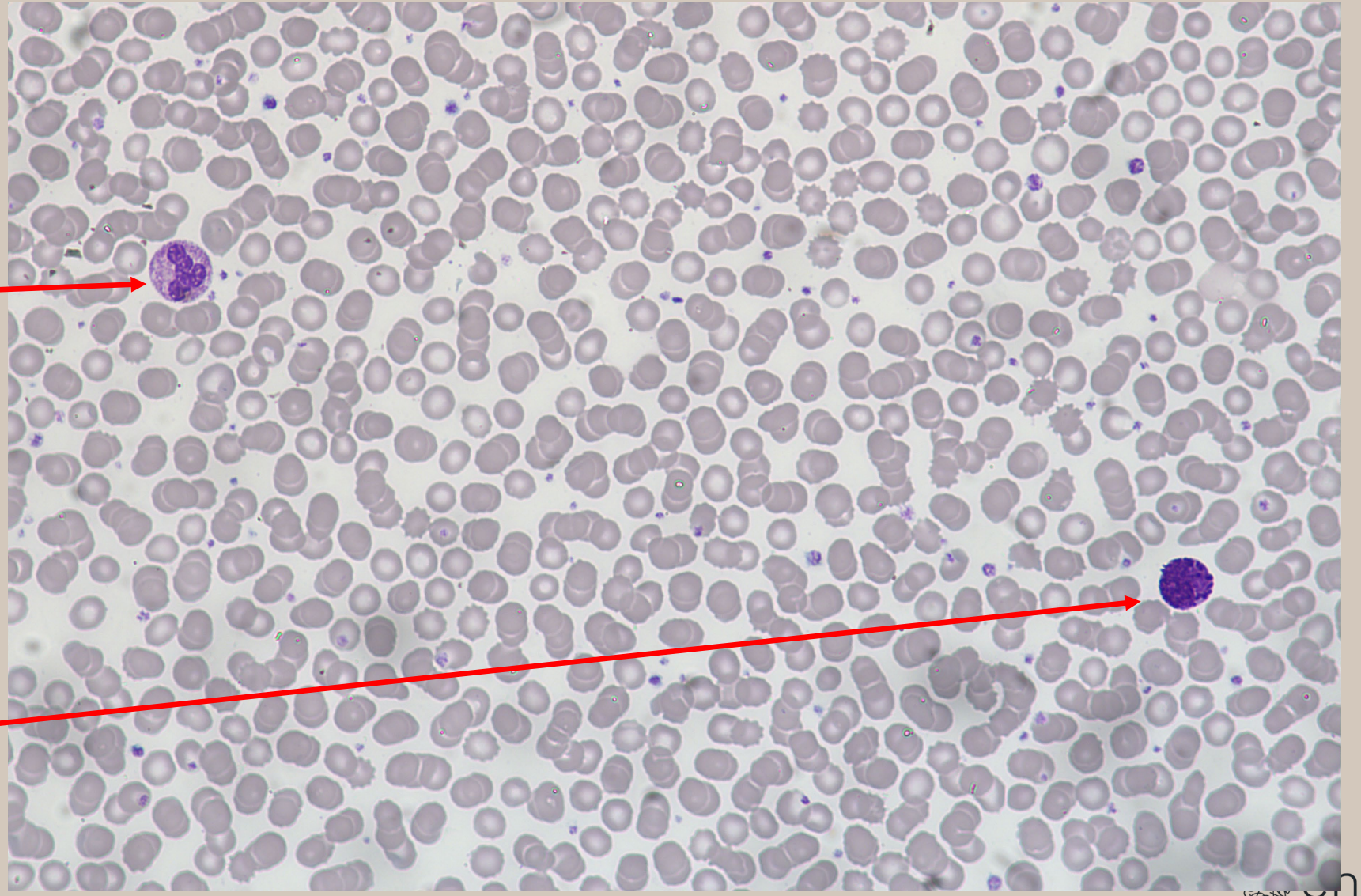
WCC 8.5 X10-9/l
Neutrophils 3.52 x10-9/l
Eosinophils 0.68x10-9
Basophils 1.61x10-9/l
Monocytes 1.39x10-9/l
Lymphocytes 1.3x10-9/l

Platelets 473



Courtesy of Dr Alesia Khan, Leeds Teaching Hospital

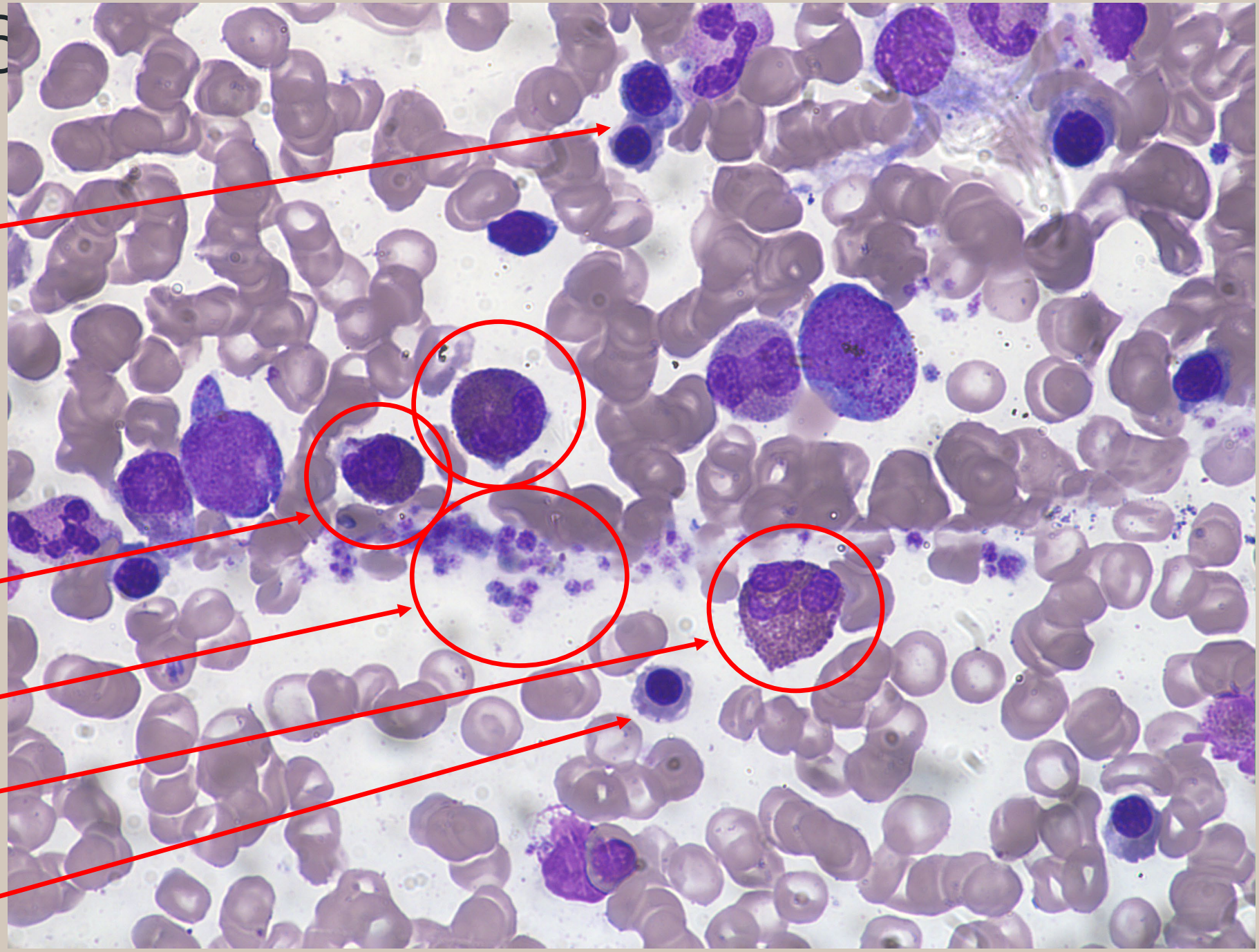
Peripheral blood x 40

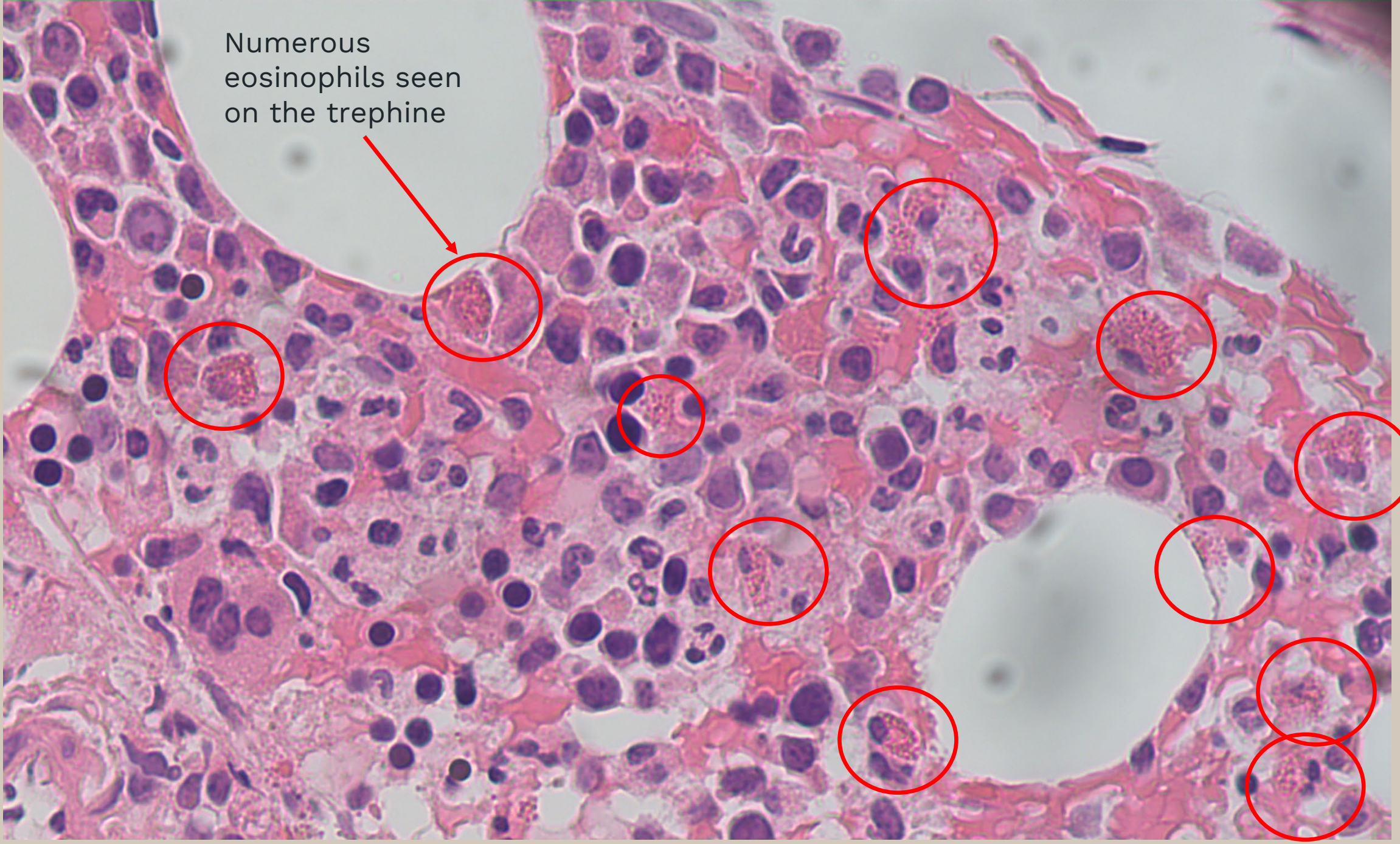


Normal mature neutrophils, numerous basophils but no significant eosinophilia.

Courtesy of Dr Alesia Khan, Leeds Teaching Hospital

Aspirate x 6


Dyserythropoiesis
Eosino-basophilic prescursors
Platelet drifts with large forms
Eosinophil
Dyserythropoiesis – cytoplasmic vacuolation



Courtesy of Dr Alesia Khan, Leeds Teaching Hospital

Case 2: Repeated BMB (2024)

Peripheral Blood Smear: The peripheral blood shows mature neutrophils with scanty left shift, 8% ($0.68x10^9$) eosinophils and 19% ($1.62x10^9$) basophils by morphology. There are no blasts.

BM Aspirate: There are small megakaryocytes seen with numerous small platelet drifts. There are occasional basophils and scattered eosinophils. There is a borderline excess of basophilic and eosinophilic precursors. There are no excess myeloid blasts, 2% by morphology seen. There is maturing granulopoiesis with some dysplasia, small promyelocytes and some hypogranular neutrophils. There is mild dyserythropoiesis with some binucleated forms and some occasional cytoplasmic vacuolation. There are no ring sideroblasts. There are no excess lymphocytes.

BM Trephine: It is normocellular for age. There is maturing trilineage hematopoiesis, with normal proportions of granulopoiesis and erythropoiesis. There are numerous scattered small monolobated megakaryocytes. There are no enlarged forms or clustering. There is no fibrosis, reticulin is grade 0. There are no mast cells. There are scattered eosinophils throughout. There are moderate numbers of macrophages. Plasma cells are not increased. There are no excess CD34 blasts. There is no T or B cell infiltrate on immunohistochemistry.

Case 2: Additional Studies

- Repeated Gene Panel: *ASXL1 VAF* 30%, *SETPB1* 44% and *STAT5B* 47%. New CBL at 10% noted.
- High-sensitive MPN NGS panel showed **no variants** in *JAK2* (exon 12 and 14 (including the p.(Val617Phe) hotspot)), *CALR* (exon 9) *MPL* (exon 10) and *CSF3R* (exon 14). LOD 1%.
- RNA Fusion Panel: No fusions were detected.
- SNP array has shown a 84.8Mb **LOH** 7q11.23-7q36.3 (containing CUX1, LAMB4, LUC7L2, BRAF, EZH2, KMT2C),

Q4) What is your diagnosis?

1. Chronic Myeloid Leukemia (CML)
2. Primary Myelofibrosis
3. AA relapse
4. MDS
5. MDS/MPN with STAT5B related eosinophilia

Q4) What is your diagnosis?

1. Chronic Myeloid Leukemia (CML)
2. Primary Myelofibrosis
3. AA relapse
4. MDS
5. MDS/MPN with STAT5B related eosinophilia

Case 2: Diagnosis

- The features are not supportive of a typical MPN(polycythemia vera), but are most consistent with an MDS/MPN unclassified or could be referred to as MDS/MPN with eosinophilia.
- *STAT5B* is driver mutation in a subgroup of chronic myeloid neoplasms, preferentially promoting a proliferation of eosinophils and basophils[1].

1. Cameron Yin, C. et al. Haematologica, June 2024

Q5) How would you treat this patient?

1. Clinical trial.
2. Cyclosporine + eltrombopag.
3. Allogeneic Stem Cell Transplantation.
4. Best supportive care.
5. Watch and wait and repeat BMB next year.

Q5) How would you treat this patient?

1. Clinical trial.
2. Cyclosporine + eltrombopag.
3. Allogeneic Stem Cell Transplantation.
4. Best supportive care.
5. Watch and wait and repeat BMB next year.

Case 2: Treatment

- As there is a clear evidence for clonal evolution in this patient, she is now being reviewed for allogenic HSCT.

Q6) How would you control her symptom while awaiting HSCT?

1. Aspirin 75mg OD
2. A trial with Hydroxycarbamide
3. Venesection
4. Prednisolone

Q6) How would you control her symptom while awaiting HSCT?

1. Aspirin 75mg OD
2. A trial with Hydroxycarbamide
3. Venesection
4. Prednisolone

Case 2: Treatment

- The aim of treatment at this stage is to control symptoms and reduce the need for venesection while waiting for HSCT. Therefore, a trial with hydroxycarbamide is the best available option in this patient.

Case 2: conclusions

- While the chance of response to immunosuppressive treatment (IST) in immune mediated AA is high, patient may relapse or progress to myeloid malignancies.
- Unanswered questions:
    - Was she a Hypoplastic MDS from the beginning?
    - Should have been transplanted back in 2005?

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