EHA-TSH Hematology Tutorial on Immune Hematological Disorders

Tutored Clinical Case

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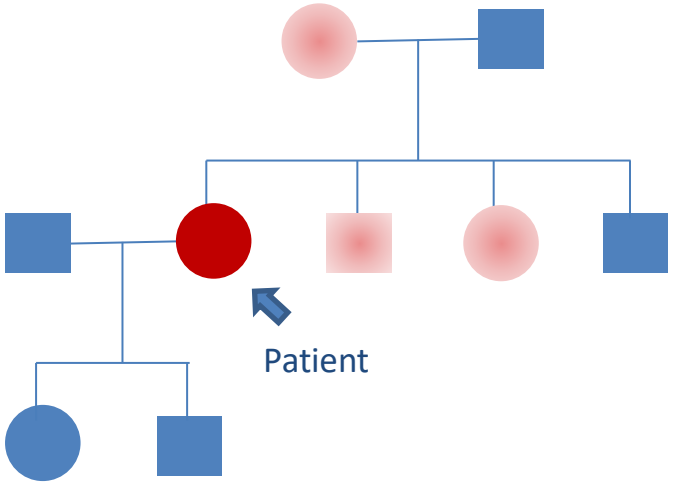
Clinical history

- A 36-year-old woman came in the Outpatient Clinic for the investigation of severe neutropenia initially identified 2 years previously.
- In her past medical history she mentioned hyperthyroidism diagnosed 5 years previously – she had receiving propylthiouracil for 3 years with it being discontinued 2 years previously – normal thyroid function since then.
- Frequent upper respiratory and skin infections that resolved with antibiotic therapy at home – pneumonia one year previously that needed hospitalization
- No current medication.
- Unremarkable physical examination.

Family History



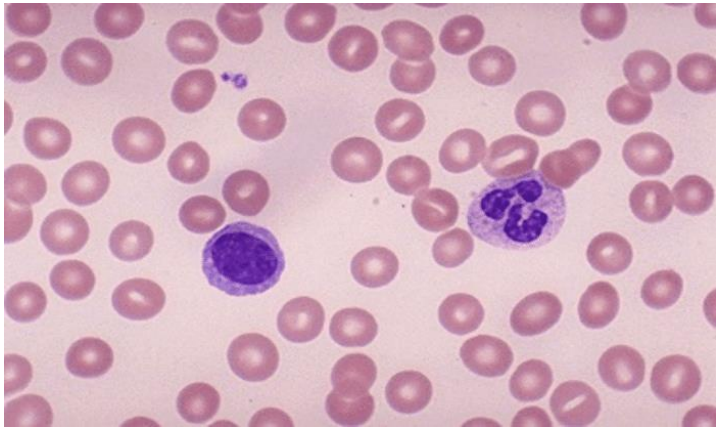
Mild neutropenia
Neutrophils 1.0 – 1.8 x 10^9/l

Severe neutropenia
Neutrophils 0.2 x 10^9/l

Normal neutrophils

Laboratory findings

- WBC 2.1 x 10^9/l
  - Neutrophils 0.2 x 10^9/l,
  - Lymphocytes 1.3 x 10^9/l,
  - Monocytes 0.5 x 10^9/l,
  - Eosinophils 0.1 x 10^9/l
- Hct 0.42 l/l, Hb 139 g/l, RBC 4.86 x 10^{12}/l
- MCV 87.2 fl, MCH 28.6 pg, MCHC 327 g/l
- Platelets 196 x 10^9/l
- ESR 20 mm/h
- Normal blood film



Normal blood film of another individual

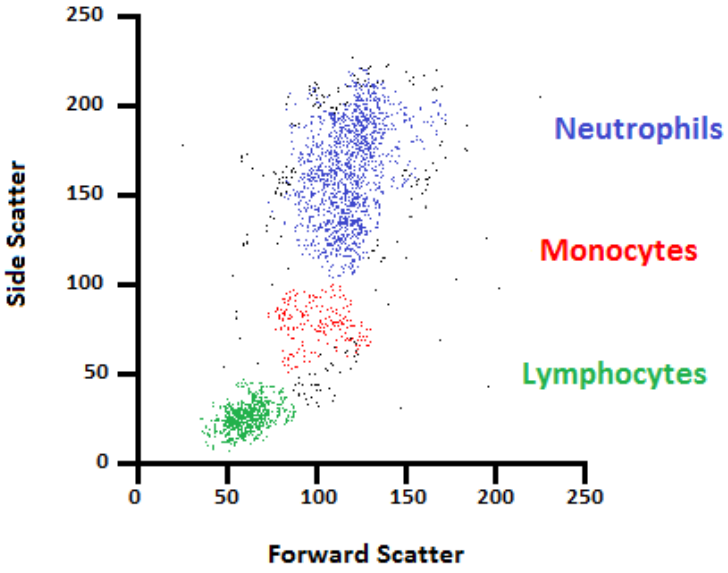
Source: web

Laboratory findings

- Biochemistry: normal
- Immunoglobulin levels: normal
- Antinuclear activity (ANA), anti-DNA antibodies, rheumatoid factor: negative
- Virology tests: negative
- Thyroid hormones: normal
- Flow cytometric analysis of PB lymphocytes

  $CD3^+$: 80%, $CD4^+$: 41%, $CD8^+$: 39%, $CD19^+$: 9%,

  $CD3^+/CD8^+/CD57^+$: 5%, $CD3^-(CD16/CD56)^+$: 10%

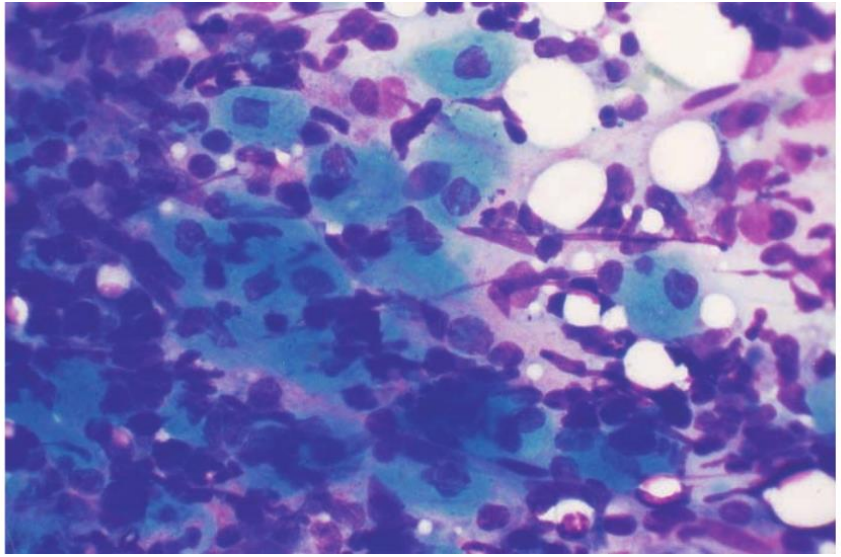


A typical flow cytometry scattergram, from:

https://www.labome.com/method/Flow-Cytometry-A-Survey-and-the-Basics.html

Bone marrow aspirate

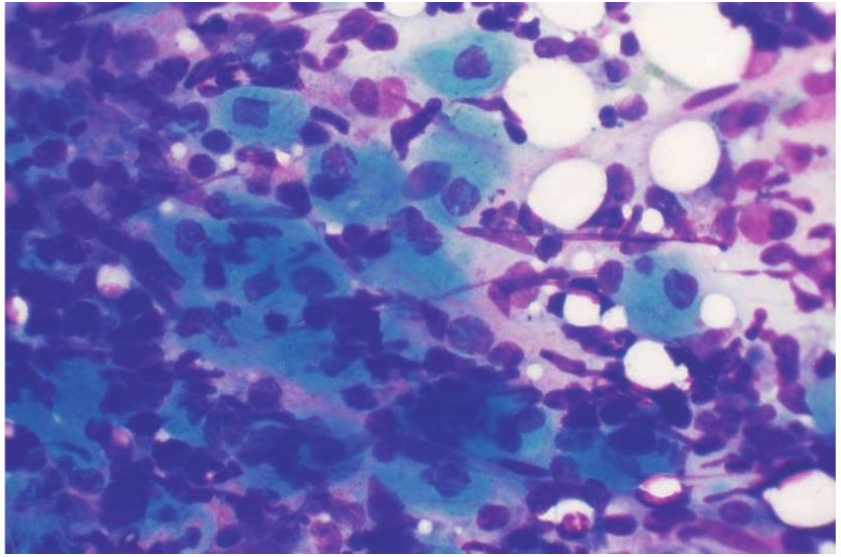
- Increased cellularity
- Normal erythroid series
- Hyperplastic myeloid series with normal maturation - a mild shift to the left (increased number of myelocytes/metamyelocytes)
- Normal megakaryocytes
- Foci of sea-blue histiocytes



Sea-blue histiocytes, from: Papadaki HA *et al*. (2002) British Journal of Haema

Bone marrow biopsy

- Increased cellularity (85%)
- Hyperplastic myeloid series and slightly decreased erythroid series. Ratio M:E ≈ 8:1
- Normal megakaryocyte series
- Disperse foci of large PAS (+) macrophages ($CD68^{+}$) with deep blue foamy cytoplasm (sea-blue histiocytes)



From: Papadaki HA *et al*. (2002) British Journal of Haematology, 118, 931

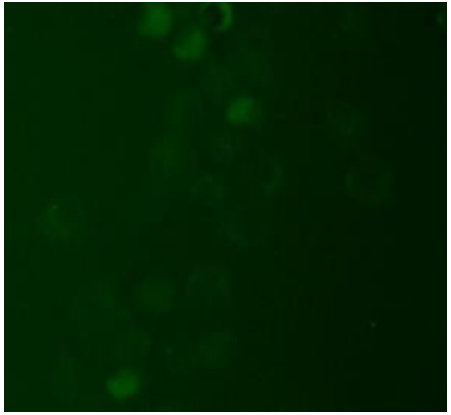
Other laboratory findings

- Karyotype: normal
- Enzymic studies to exclude lipid storage diseases
  - Chytotriosidase activity: normal
  - Sphyngomyelinase activity: normal
- Ultrasound of abdomen
  - Liver: normal
  - Spleen: 12 cm x 9 cm x 4.5 cm (normal)

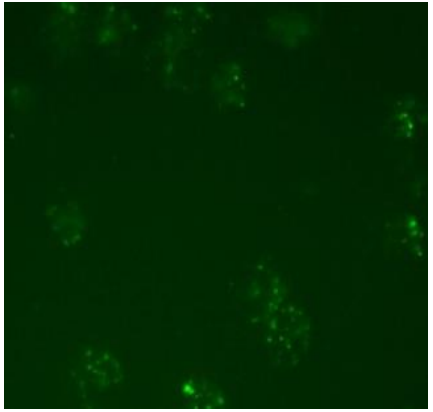
Granulocyte antibody screening

- Granulocyte Immunofluorescence Test (GIFT)
  - 3 of 4 panel cells positive
- Granulocyte Agglutination Test (GAT)
  - 4 of 4 panel cells positive
- Lymphocyte Immunofluorescence Test (LIFT)
  - 4 of 4 panel cells negative
- Monoclonal antibody-specific immobilization of granulocyte antigens (MAIGA)
  - Identification of a strong positive anti-FcRIIIb antibody

Granulocyte Immunofluorescence Test (GIFT)

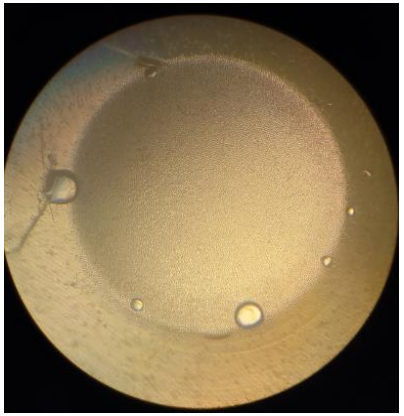


Negative control

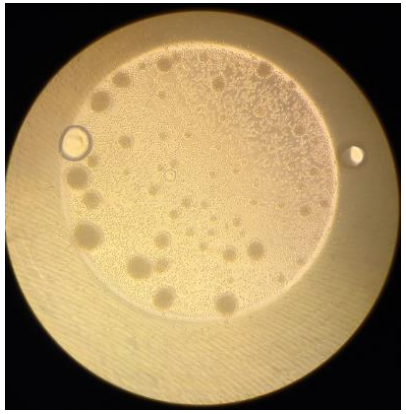


Patient's sample

Granulocyte Agglutination Test (GAT)



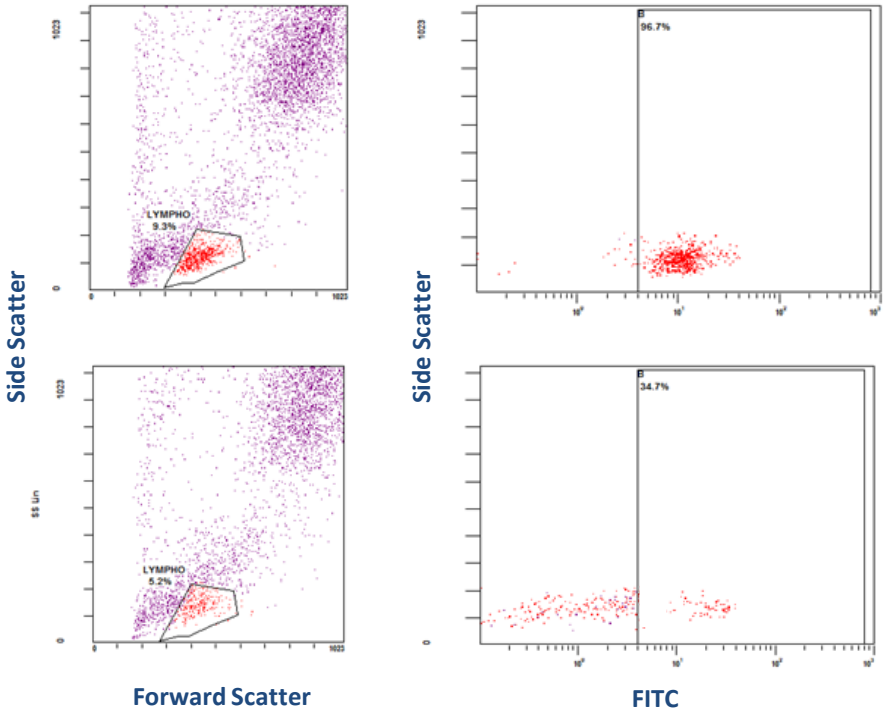
Negative control



Patient's sample

Haemopoiesis Research Lab, University of Crete, Greece

Lymphocyte Immunofluorescence Test, LIFT



Positive control

Patient's sample

Haemopoiesis Research Lab,
University of Crete, Greece

Final diagnosis

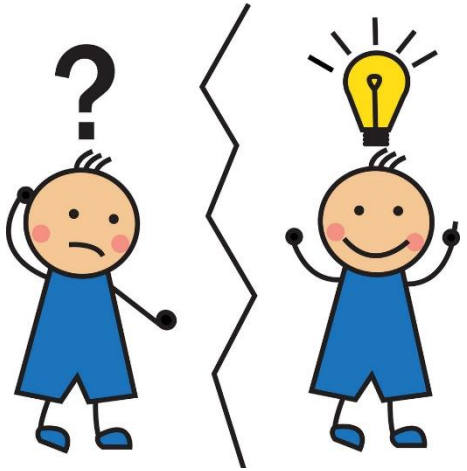
- Primary autoimmune neutropenia (AIN)

  But….

  how do you explain the presence of sea-blue histiocytes in the BM ?

Final diagnosis

- Primary autoimmune neutropenia (AIN)
- We hypothesize that the antibody-mediated increased granulocyte destruction leads to accumulation of high amounts of lipids in BM macrophages, which exceed their catabolic capacity and result in the formation of lipid-laden sea-blue histiocytes.



Papadaki HA *et al*. (2002) British Journal of Haematology, 118, 931

Treatment of the patient

Lenograstim (G-CSF)

2 µg/kg 2 times a week subcutaneously

WBC 4.6 x 10^9/l

Neutrophils 2.6 x 10^9/l

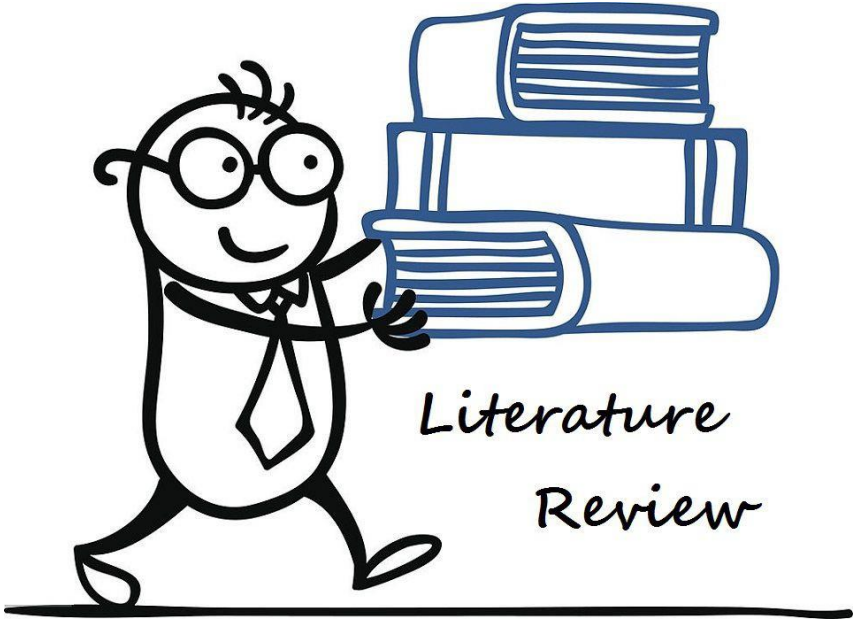
Lymphocytes 1.4 x 10^9/l

Monocytes 0.5 x 10^9/l

Eosinophils 0.1 x 10^9/l

Primary AIN in adults

Discussion, Treatment



Discussion

- AIN in adults can be divided into primary and secondary.
  - Primary AIN is not a common condition in adults.
  - Secondary AIN is usually associated with other autoimmune disorders, haematological and non-haematological malignancies and drug exposure.
- AIN may be associated with an increased risk of infection, particularly if absolute neutrophil counts are lower than 0.5×10^9/l.
  - Bacterial infections such as intermittent stomatitis and gingivitis, perirectal abscess and cellulitis are more common than pneumonia and septicaemia.
  - Fungal infections are generally unusual, but oral candidiasis is common.
  - There is usually no increased risk of viral or parasitic infections.

Akhtari M *et al*. (2009) Autoimmunity Reviews, 9, 62-66

Treatment

- G-CSF: first line treatment, starting dose of 5 µg/kg/day subcutaneously.
  - Stimulates proliferation and maturation of neutrophil progenitor cells → increases production and release of mature neutrophils into the circulation.
  - Demarginates neutrophils → clearance of significant amounts of neutrophil auto-antibodies from the circulation.
  - Reduces neutrophil apoptosis.
  - Induces neutrophils to shed FcγRIIIb from their plasma membrane → clearance of neutrophil autoantibodies from the blood circulation.

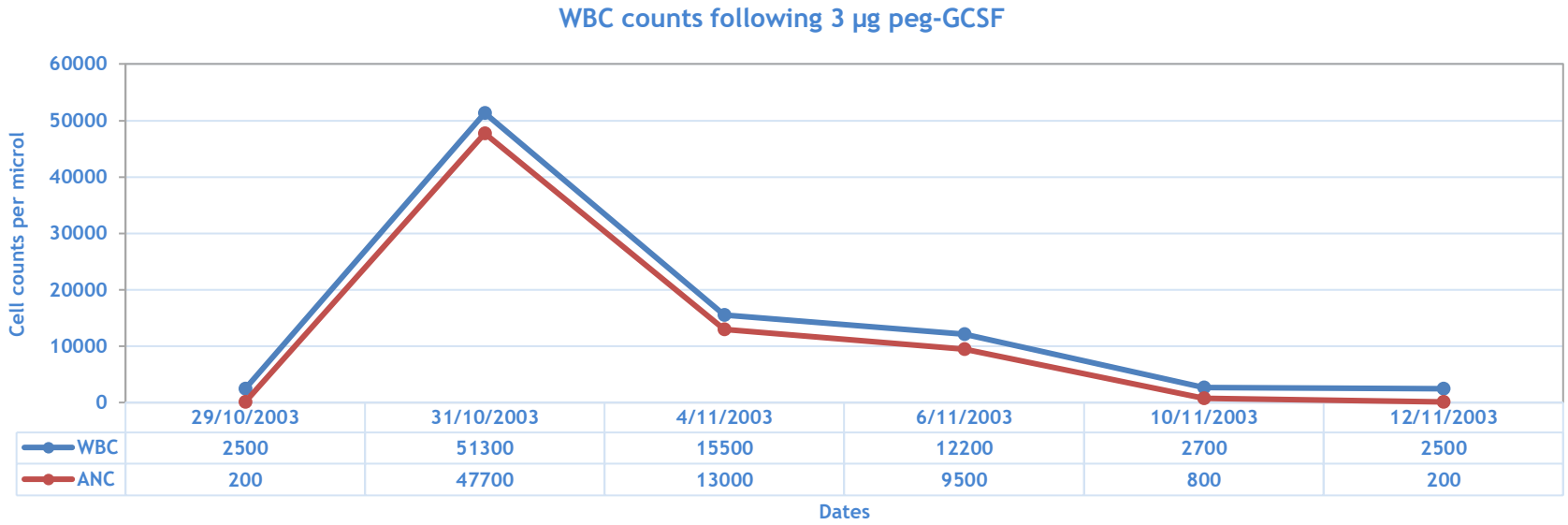
Akhtari M *et al*. (2009) Autoimmunity Reviews, 9, 62-66

Treatment *(continued)*

- G-CSF is usually given during infections in combination with antibiotics.
- Continuous G-CSF for patients with recurrent infections or mucosal erosions.
- Use the minimum dose/frequency to maintain neutrophil counts in a range to prevent symptoms.
- Avoid pegylated G-CSF in AIN due to severe and prolonged bone pain and a risk for excessive increase in neutrophils.
- In contrast to patients with severe congenital neutropenia, there is no risk of leukaemia development in adults with AIN receiving long-term treatment with G-CSF.

Gibson C & Berliner N. (2014) Blood, 124:1251-1258

Patient's WBC and neutrophil count (ANC) following peg-G-CSF treatment



Abbreviations:
WBC, white blood cell counts
ANC, absolute neutrophil counts

Treatment AIN *(continued)*

- Splenectomy: in the era before G-CSF; no encouraging results.
- Corticosteroids: limited effect.
- Ciclosporin or sirolimus: case reports showing response.
- Intravenous immunoglobulin (IVIG): Transient effect in 50% of patients.
- Campath-1H (alemtuzumab): rare responses in patients with severe AIN, who do not respond to conventional treatments or who have a chronic relapsing course and suffer life-threatening infections.
- Rituximab: no effect

Akhtari M *et al*. (2009) Autoimmunity Reviews, 9: 62-66
Marsh JC & Gordon-Smith EC. (2001) Cytotherapy, 3: 189–195.

Representative Literature

- Akhtari M, Curtis B, Waller EK. Autoimmune neutropenia in adults. Autoimmun Rev. 2009;9(1):62-6. doi: 10.1016/j.autrev.2009.03.006.
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- Bux J. Human neutrophil alloantigens. Vox Sang. 2008;94(4):277-85. doi: 10.1111/j.1423-0410.2007.01031.x. Epub 2008 Jan 16.
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