INTERACTIVE CLINICAL CASE
How to Approach Neutropenia

EHA-ISHBT Hematology Tutorial

March 1-3, 2024
Hyderabad, India

F Fioredda
Unita' di Ematologia
IRCCS
Istituto G Gaslini

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PERSONAL AND FAMILY HISTORY

Caucasian boy, born in 2005; weight at birth 3,000 g, healthy since 2014 except for mild asthma *(no available WBC)* and recurrent bronchitis

Psoriasis and thyroiditis in family history, no parental consanguinity

PAST HISTORY

June 2014 Abdominal pain (>> right iliac fossa), diarrhea and vomiting

Ultrasound (US) Abdomen: mild splenomegaly, lymphadenopathy sites: ileum and caecum.
WBC 3.0 x 10^9/L, absolute neutrophil count (ANC) 0.41 x 10^9/l , lymphocytes 1.5 x 10^9/l, Hb 130 g/l, Plt 331 x 10^9/l
C-reactive protein (CRP) 4.13 mg/dl (ref value <0.46 mg/dl)

Following months:
Persistence of intermittent intestinal symptoms & mild splenomegaly

October 14 WBC 3.7 x 10^9/l ANC 1.47 x 10^9/l, Lymphocytes 1.6 x10^9/l, Hb 142 g/l, Plt 254 x 10^9/l
November 14 WBC 2.5 x 10^9/l, ANC 0.4 x 10^9/l, Lymphocytes 1.75 x 10^9/l , Hb 138 g/l, Plt 212 x 10^9/l

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«OUR» HISTORY

First observation in our ward Dec 2015

WBC 2.35 x 10^9/L, ANC 0.33 x 10^9/L, Lymphocytes 1.38 x 10^9/L , Eosinophils 0.16 x 10^9/L Hb 16 g/L, Plt 211 x 10^9/L

Ferritin, transferrin, plasma iron, vitamin B12, thyroid function normal
Antinuclear activity (ANA), Extractible Nuclear Antigen, direct antiglobulin test (DAT), indirect antiglobulin test (IAT), anti-thrombopoietin, anti-thyroglobulin negative

IgA-anti-gliadin antibodies (AGA), tissue transglutaminase (TG2) and endomysium (EMA) testing negative

Ab against neutrophils negative

IgG 1.01 g/l
IgA 0.75 g/l
IgM 0.82 g/l

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BONE MARROW ASPIRATE (January 16)

Bone marrow: cellularity +++

Megakaryocytes	present
M:E ratio	4:1
Myeloblasts	2%
Promyelocytes	16%
Myelocytes	29%
Metamyelocytes	28%
Band forms	20%
Mature neutrophils	5%

BM Karyotype 46,XY

BM clonogenic assay: reduced growth of myeloid precursors

BONE MARROW BIOPSY

cellularity 70%

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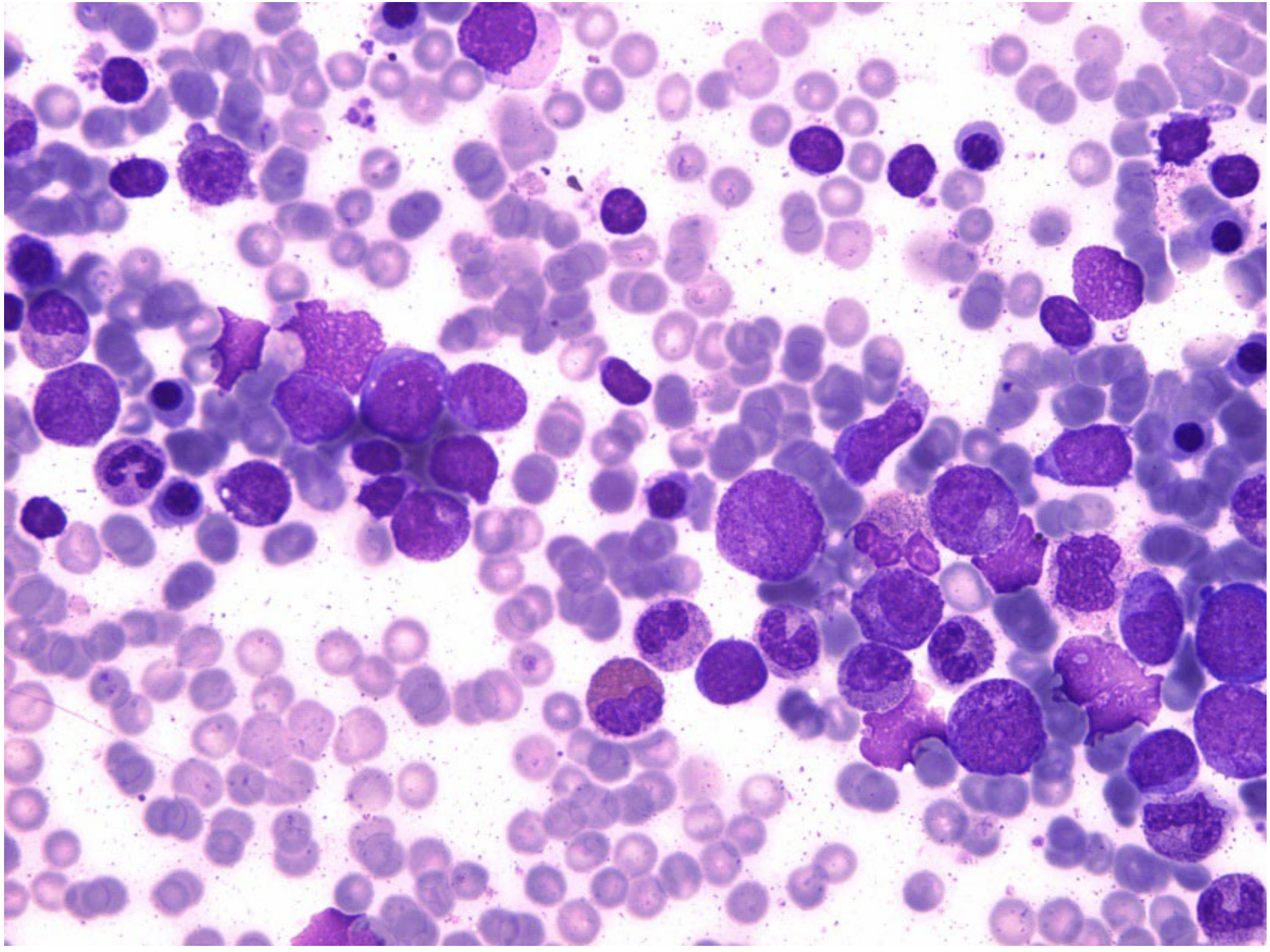

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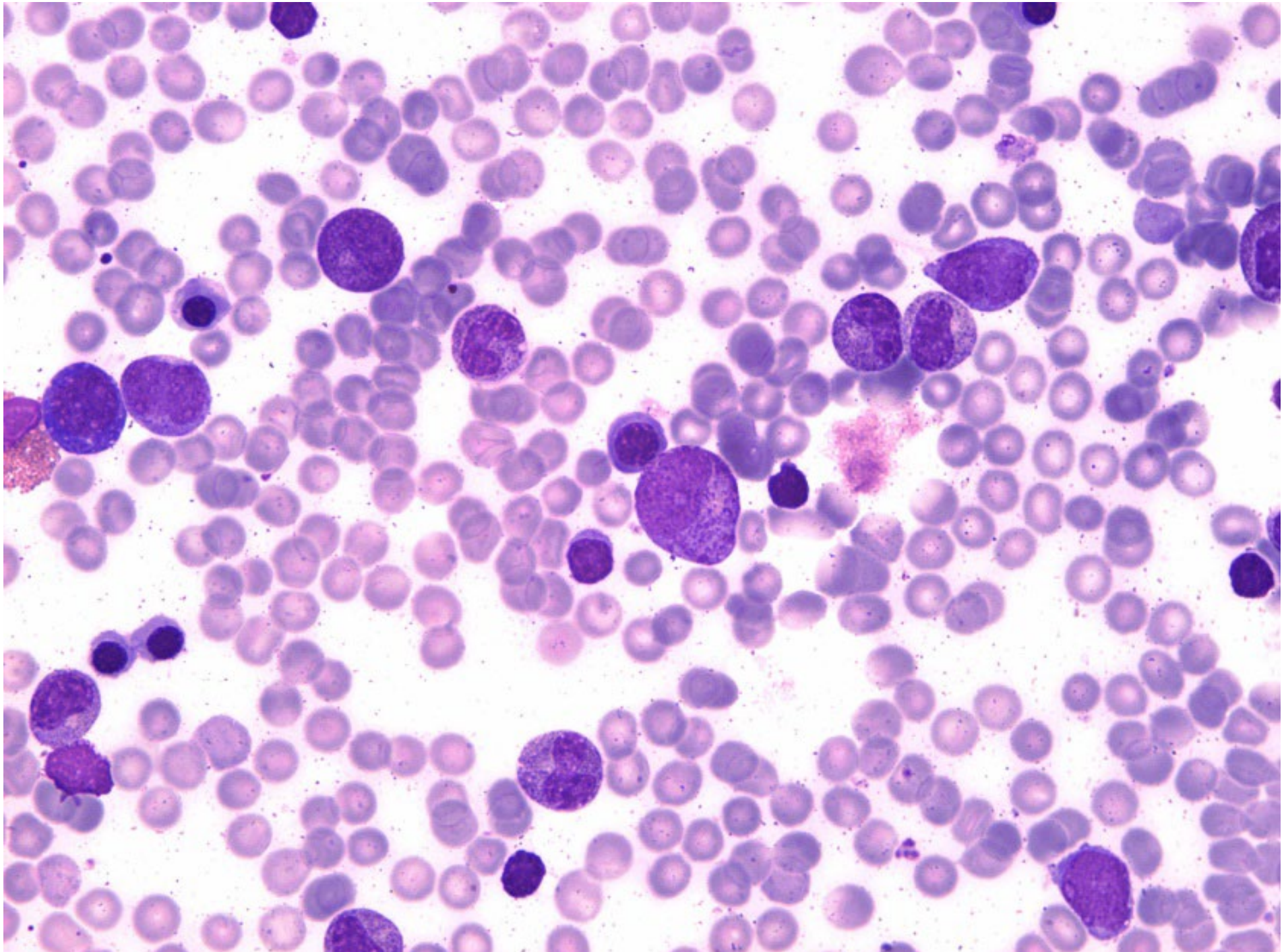

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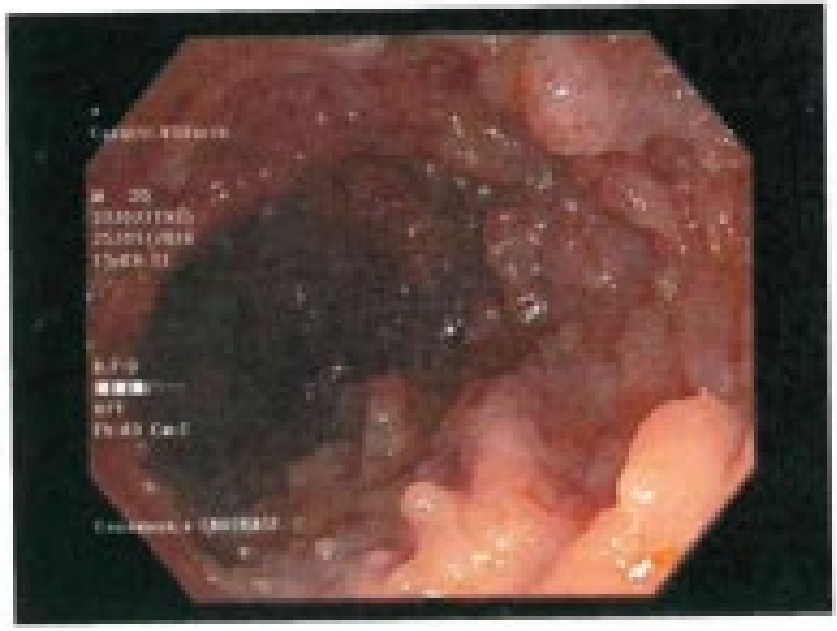
ENDOSCOPY (January 16)

-Duodenal/gastric biopsy: patchy inflammation with lymphocytic infiltration

-Colon/rectum endoscopy: macroscopic features and histology normal/negative



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IMMUNOLOGY	Basal
Total Lymphocytes x10^9/µL	1.17
CD3+	79 %
CD3+CD4+	42 %
CD3+CD8+	20 %
CD19+	13 %
CD3+HLA-DR+	10%
CD3+ TCRγδ+	14%↑
CD3+CD4+CD25 bright+ CD45-	1.3%
CD3+CD16+CD56+	1 %↓
CD3-CD16+CD56+	6.2 %↓
CD3+TCRαβ+ CD4-CD8- *of total lymphocyte*	2.3%↑
CD19+CD27+	35%

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NIH 2009 Diagnostic criteria for Autoimmune Lymphoproliferative Syndrome (ALPS)

Required

1. Chronic (> 6 months), nonmalignant, noninfectious ***lymphadenopathy or splenomegaly or both***
2. Elevated $CD3^{+}TCR\alpha\beta^{+}CD4^{-}CD8^{-}$ **DNT cells**

Accessory

Primary

1. **Defective lymphocyte apoptosis**
2. **Somatic or germline pathogenic mutation** in *FAS*, *FASLG*, or *CASP10*

Secondary

1. > sFASL levels or > interleukin-10 levels or > vitamin B12 levels or > interleukin-18 levels
2. Typical hystology
3. Autoimmune cytopenias (hemolytic anemia, thrombocytopenia, or neutropenia) and Hypergammaglobulinemia
4. Family history of a nonmalignant/noninfectious lymphoproliferation with or without autoimmunity

definitive diagnosis ***both required criteria + one primary accessory criterion***
probable diagnosis ***both required criteria+ one secondary accessory criterion***

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HISTORY UPDATE & results 2016 /2017

Ab against neutrophils
#2 sample negative
#3 sample positive!!
Resolution of splenomegaly
FAS apoptosis functional test negative
Negativity of coeliac markers (3 times)
Resolution of splenomegaly

Clinical symptoms: propensity to skin infection

Paucisymptomatic COVID-19 infection in 2020

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IMMUNOLOGY	Basal	Intermediate	Last FUP
Total Lymphocytes x10^9/µL	1.17	1.00	1.33
CD3+	79 %	77%	74%
CD3+CD4+	42 %	43%	43%
CD3+CD8+	20 %	21%	22%
CD19+	13 %	15%	12.6%
CD3+HLA-DR+	10%	9.5%	15%
CD3+ TCRγδ+	14%↑	11% ↑	12.1%↑
CD3+CD4+CD25 bright+ CD45-	1.3%	0.5% ↓	0.1%↓
CD3+CD16+CD56+	1 %↓	0.4%↓	2.8%↓
CD3-CD16+CD56+	6.2 %↓	7.1 % ↓	9.2%↓
CD3+TCRαβ+ CD4-CD8- (Lymph tot)	2.3%↑	1.8%	1.2 %
CD19+CD27+	35%	40%	28%

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Key points

Chronic Neutropenia

Chronic gastrointestinal (GI) symptoms

Positivity of Ab against Neutrophils

HOW TO PROCEED

Diagnosis and Management

How I can manage propensity to infections?

Is the posivity of Ab against Neutrophils enough to define the diagnosis?

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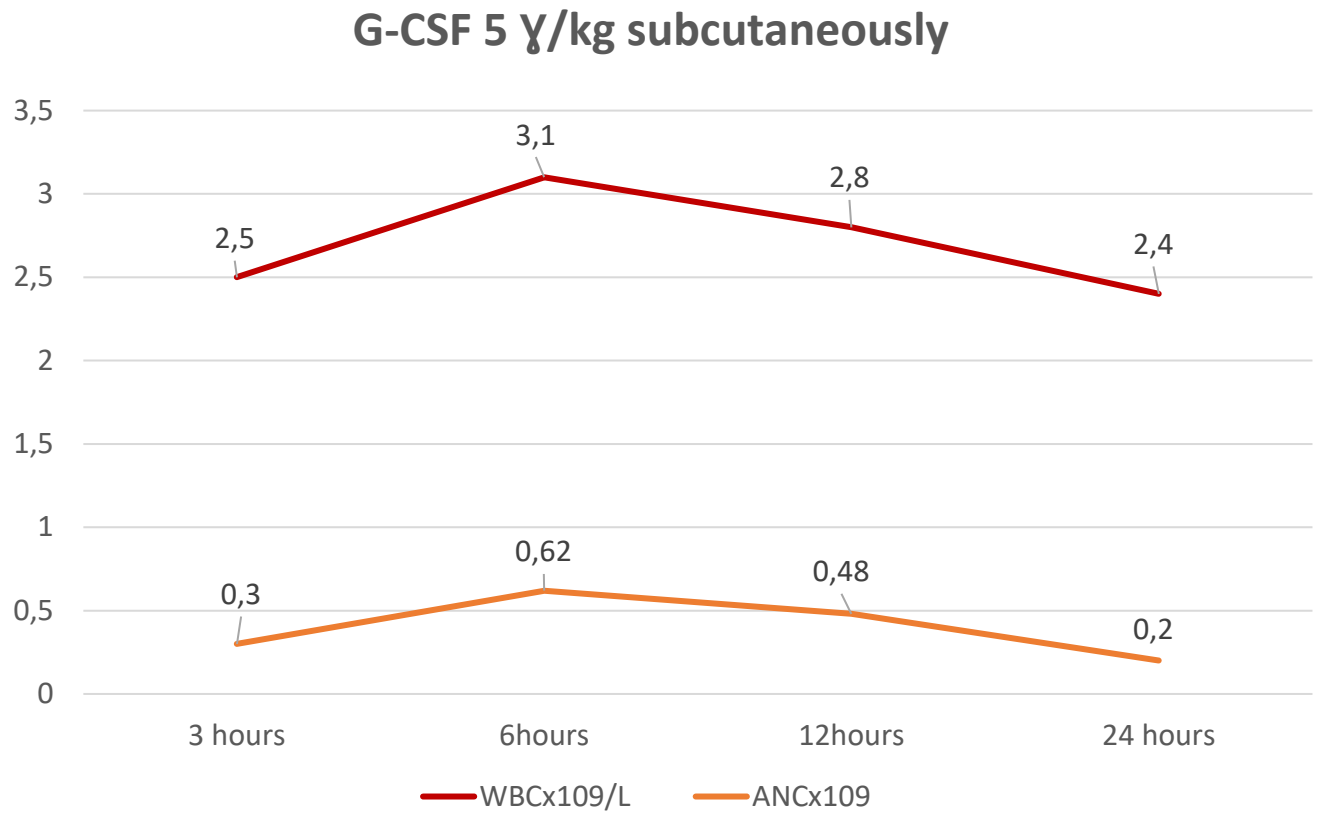
G-CSF test



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B maturation profile

Transitional (CD27-CD10++CD38++)	**0.5↓**
Naive (CD27-CD10+-CD38+-IgD+)	**54.9↓**
Marginal zone (CD27+IgD+IgM+)	**24.6↑**
Switched memory (CD27+IgD-IgM-)	**5.8↓**
Pre-switched memory (CD27+IgD-IgM+)	0
IgD memory (CD27+IgD+IgM-)	0
Antibody-secreting cells	**0.5↓**
Double Negative (DN) (CD27-IgD-)	**13.5↑**
B memory (%)	31.3

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GENETIC PANEL

NGS (Next Generation Sequencing) panel of Bone Marrow Failure & PIRD (Primary Immunodysregulation disorders)

AIRE, CARD11, CASP10, CASP8, CD19, CD20, CD40, CD40L, CD70, CTLA4, CTPS1, DCLRE1C, FADD, FAS, CECR1/ADA2, FASL, FOXP3, GATA2, GBA, GORASP1, IKZF1, IL10, IL10RB, IL2RA, ITK, KRAS, LRBA, NCKAP1L/HEM1, NEMO, NFKB1, NRAS, PIK3CB, PIK3CD, PIK3R1, PRKCD, RAG1, RAG2, RASGRP1, SOCS1, STAT1, STAT3, STAT5B, MPL, TLR8, TNFRSF13B, TNFRSF13C, MAGT1, ACD/TPP1, CTC1, DKC1, MYSM1, NAF1, NHP2, NOP10, PARN, POT1, RPL11, RPL15, RPL26, RPL27, RPL35A, RPL5, RPS10, RPS17, RPS19, RPS24, RPS26, RPS27, RPS28, RPS29, RPS7, RTEL1, STN1, TERC, TERT, TINF2, TSR2, WRAP53/TCAB1, RPS14, ERCC6L2, SRP72, TP53, C16orf57/USB1, DNAJC21, EFL1, SBDS, SRP54, ATM, BLM/RECQL3, LIG4, NBN, NHEJ1, AP3B1, BLOC1S6the, CD27, LYST, PRF1, SH2D1A, SLC7A7, STX11, STXBP2, UNC13-D, XIAP/BIRC4, ANKRD26, ASXL1, ATG2B, CD79B, DDX3X, DDX41, EOMES, ERAP1, GATA3, GSKIP, IL13, MKL1, MYD88, PVT1, RBBP6, REL, TCF3, ETV6, ACKR1/DARC, AK2, AK3, CLPB, CSF3R, CXCR2, CXCR4, DNM2, EIF2, ELA2, G6PC3, GFI1, HAX1, JAGN1, LAMTOR2, RAC2, RMRP, SEC61A1, SLC37A4, SMARCAL1, SMARCD2, STK4,s TAZ, TCIRG1, TCN2, VPS13B, VPS45, WAS, WIPF1, RAB27A, GATA1, ADAR1, CBL, CEBPA, MECOM, NPM1, RUNX1, SAMD9, SAMD9L

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CHOICE OF GENETIC METHODS

Sanger polymerase chain reaction (PCR) very suggestive clinical picture (i.e. ELANE in case of severe infections and block at promyelocytes)

Multigenes NGS panel or targeted whole exome sequencing (WES) (when the clinical picture does not suggest a specific genetic cause)

Whole Genome Sequencing (WGS)/RNA sequencing in case of negativity of the above methods

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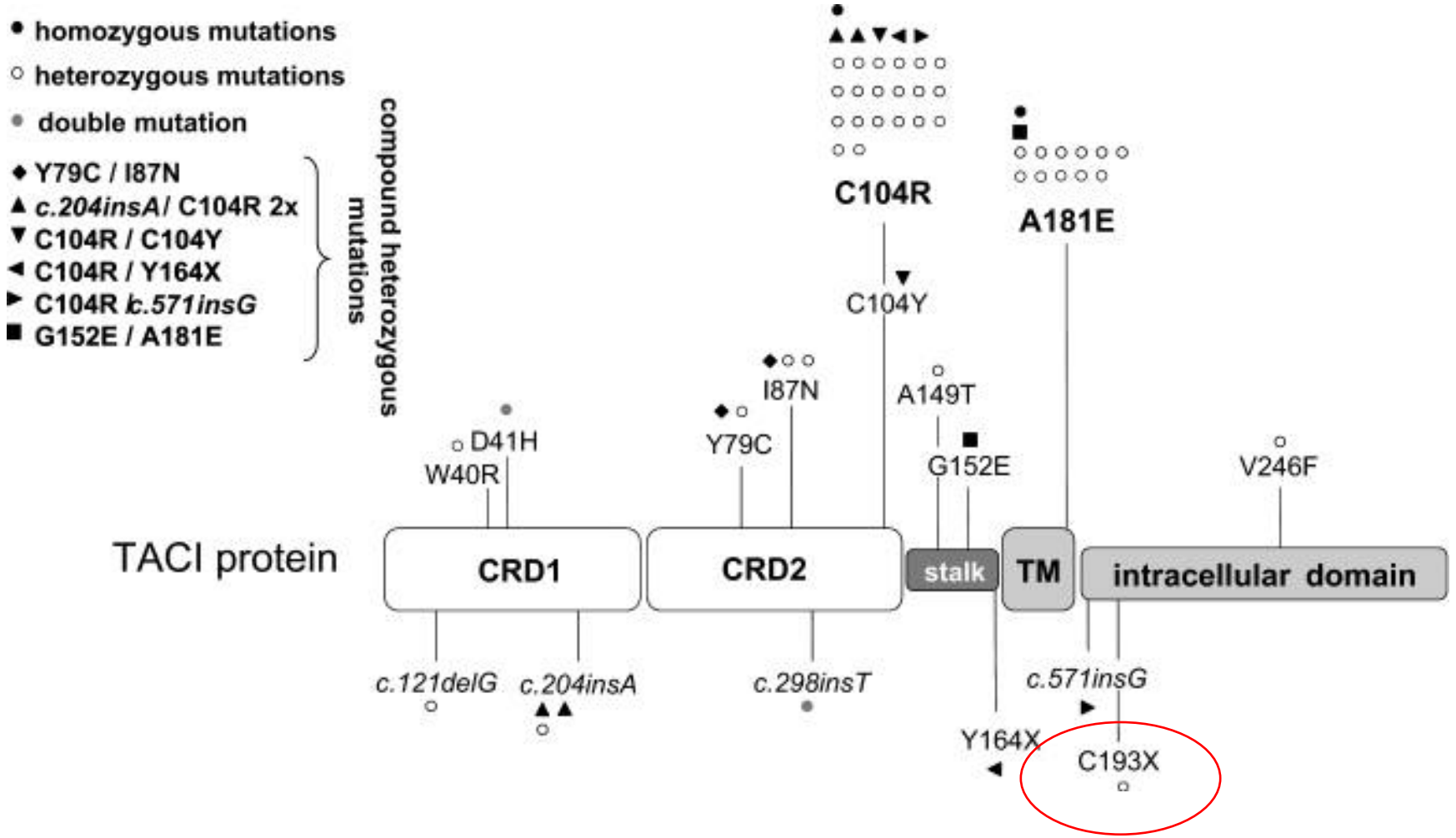
GENETIC DEFINITION

TACI (TNFRSF13B) c.579 C>A, p.Cys193Ter (heterozygous)

Varsome LP, ClinVAr P, Gnom Ad 0.0058%



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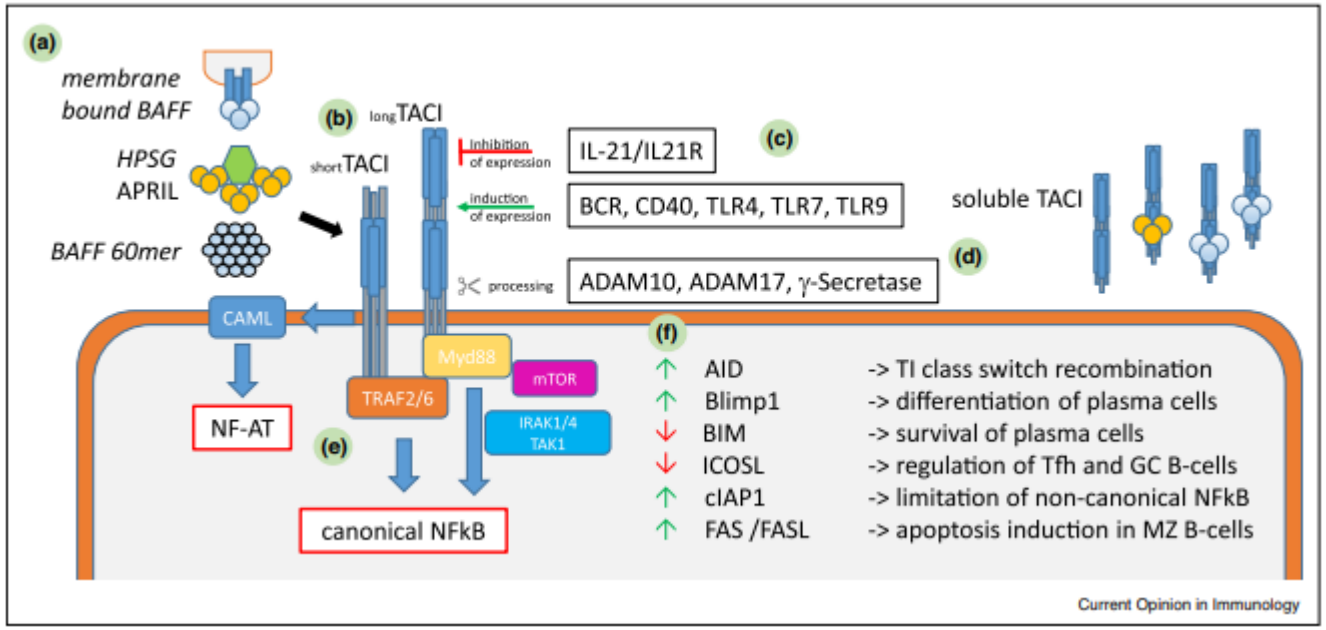
TACI (TNF(R)SF members)



Promotes T-cell-independent antibody responses/plasma cell differentiation.

Counteracts BAFF-driven B cells

SPECTRUM Benign lymphoproliferation
Autoimmunity

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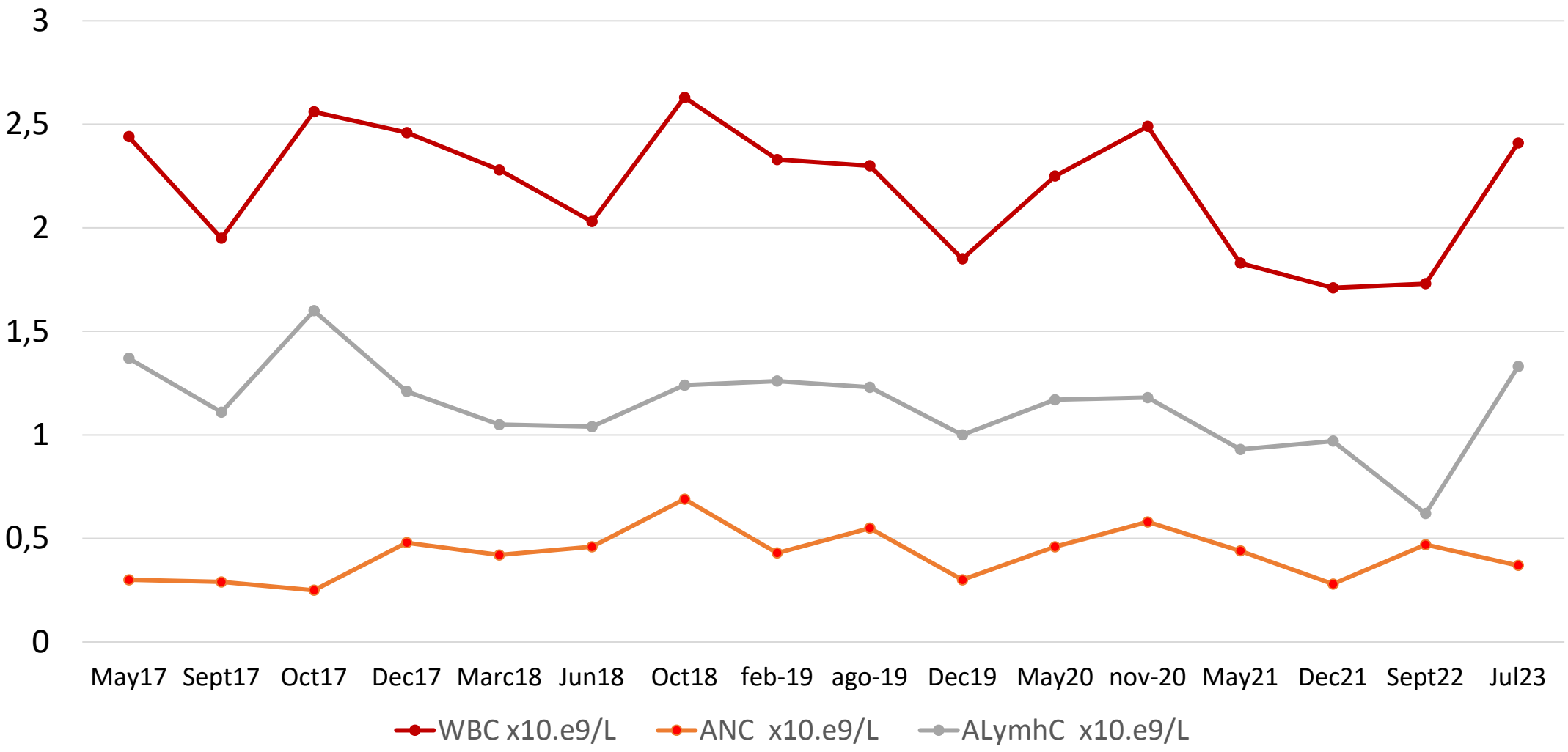
Autoimmune cytopenia, 3.34-fold higher in patients with *TACI* mutation *vs* healthy population

Salzer U. Blood. 2009, *Salzer U Curr Opin Immunol. 2021*

WBC, ANC, Lymphocyte Count over time



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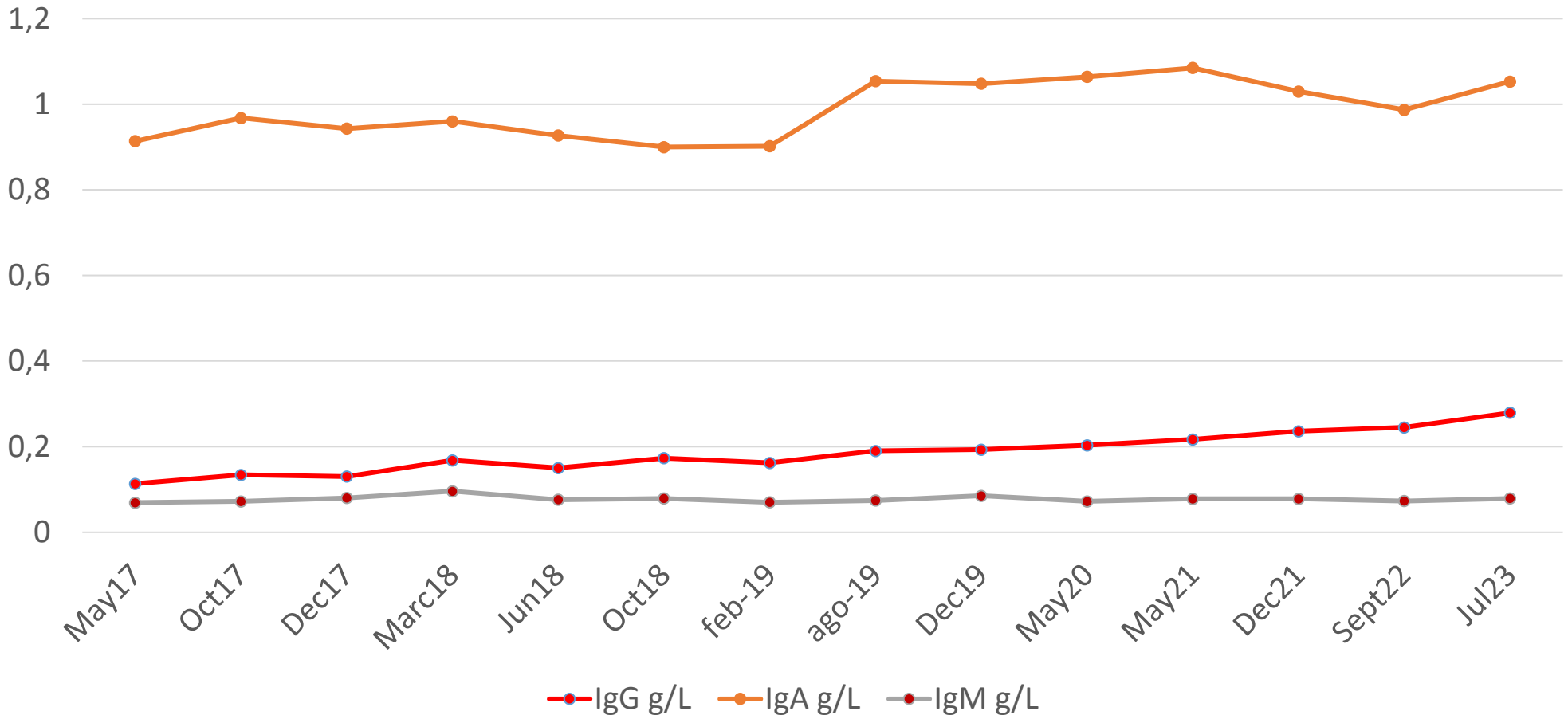
Immunoglobulin Values over time



LAB REFERENCES VALUE **IgG** 0.7-1.6 g/L, **IgA** 0.07-0.4 g/L, **IgM** 0.04-0.23

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FOLLOW UP - WHY?

Monitor the infectious burden in order to re-evaluate the need for G-CSF therapy

Check immunoglobulin values over time to establish if immunoglobulin infusion is needed

Observe any possible appearance of autoimmunity (thyroid, skin, joints etc.) or other clinical signs

Enlarge the genetic «coverage» in case of panel negativity

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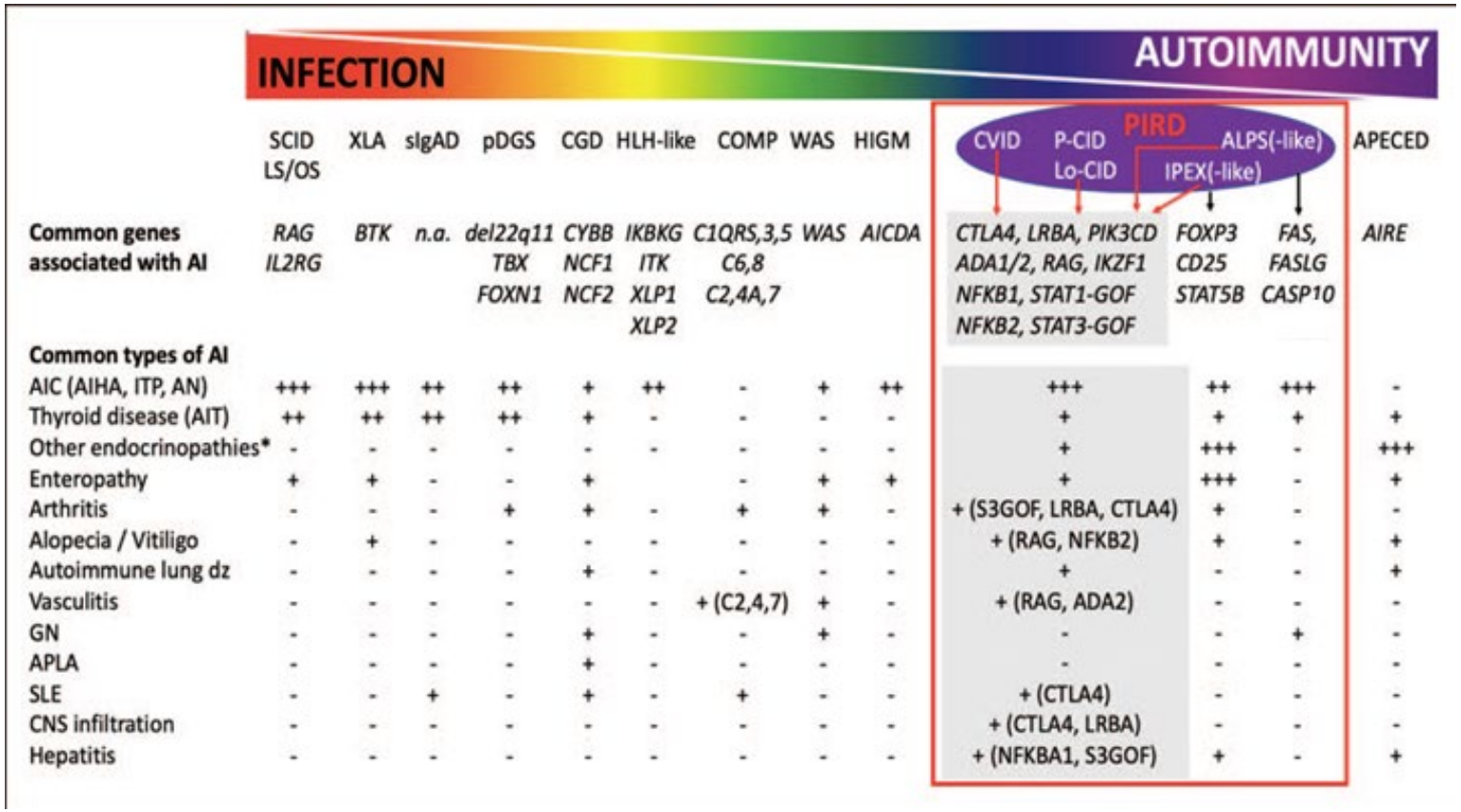
FINAL CONSIDERATION AND DIAGNOSIS 1

PIRD Primary Immune Regulatory Disorders & AUTOIMMUNE CYTOPENIA



	SCID LS/OS	XLA	sIgAD	pDGS	CGD	HLH-like	COMP	WAS	HIGM	CVID P-CID Lo-CID IPEX(-like) ALPS(-like)	APECED
Common genes associated with AI	*RAG IL2RG*	*BTK*	*n.a.*	*del22q11 TBX FOXN1*	*CYBB NCF1 NCF2*	*IKBKG ITK XLP1 XLP2*	*C1QRS,3,5 C6,8 C2,4A,7*	*WAS*	*AICDA*	*CTLA4, LRBA, PIK3CD ADA1/2, RAG, IKZF1 NFKB1, STAT1-GOF NFKB2, STAT3-GOF*	*AIRE*
Common types of AI											
AIC (AIHA, ITP, AN)	+++	+++	++	++	+	++	-	+	++	+++	-
Thyroid disease (AIT)	++	++	++	++	+	-	-	-	-	+	+
Other endocrinopathies*	-	-	-	-	-	-	-	-	-	+	+++
Enteropathy	+	+	-	-	+		-	+	+	+	+
Arthritis	-	-	-	+	+	-	+	+	-	+ (S3GOF, LRBA, CTLA4)	-
Alopecia / Vitiligo	-	+	-	-	-	-	-	-	-	+ (RAG, NFKB2)	+
Autoimmune lung dz	-	-	-	-	+	-	-	-	-	+	+
Vasculitis	-	-	-	-	-	-	+ (C2,4,7)	+	-	+ (RAG, ADA2)	-
GN	-	-	-	-	+	-	-	+	-	-	-
APLA	-	-	-	-	+	-	-	-	-	-	-
SLE	-	-	+	-	+	-	+	-	-	+ (CTLA4)	-
CNS infiltration	-	-	-	-	-	-	-	-	-	+ (CTLA4, LRBA)	-
Hepatitis	-	-	-	-	-	-	-	-	-	+ (NFKBA1, S3GOF)	+

Late-onset or profound combined immunodeficiency disorders (LoCID, P-CID)

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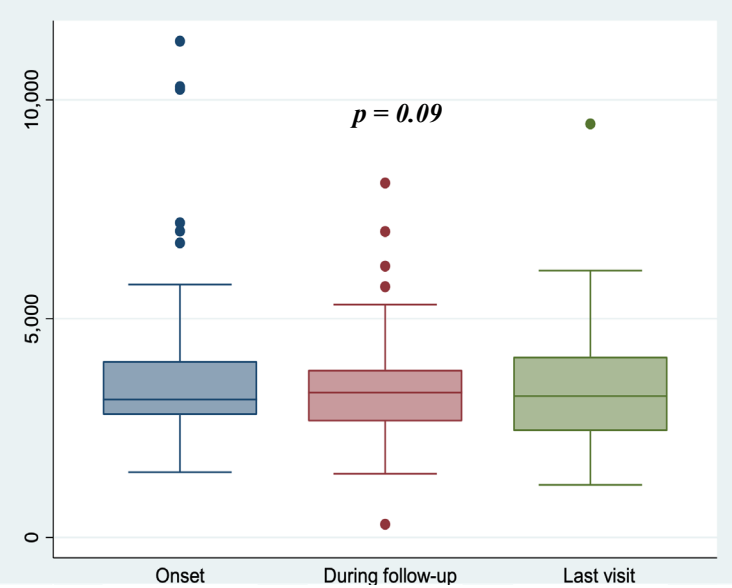
Jolan WE , Current Opinion in Immunology 2019

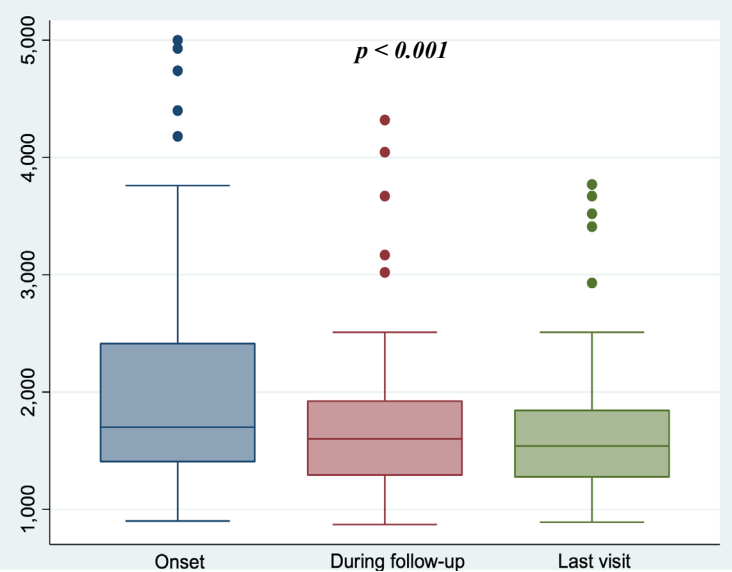
Late onset and long lasting neutropenias

Mild/silent phenotype, leukopenia, anti neutrophil antibodies +/-, possible appearance of autoimmunity, variants of immune-dysregulation

LEUCOCYTES



LYMPHOCYTES



IMMUNOLOGY

Low *CD8/B/NK cells, T regulatory, B memory*

High *γ/δ, HLA-DR+ T cells*

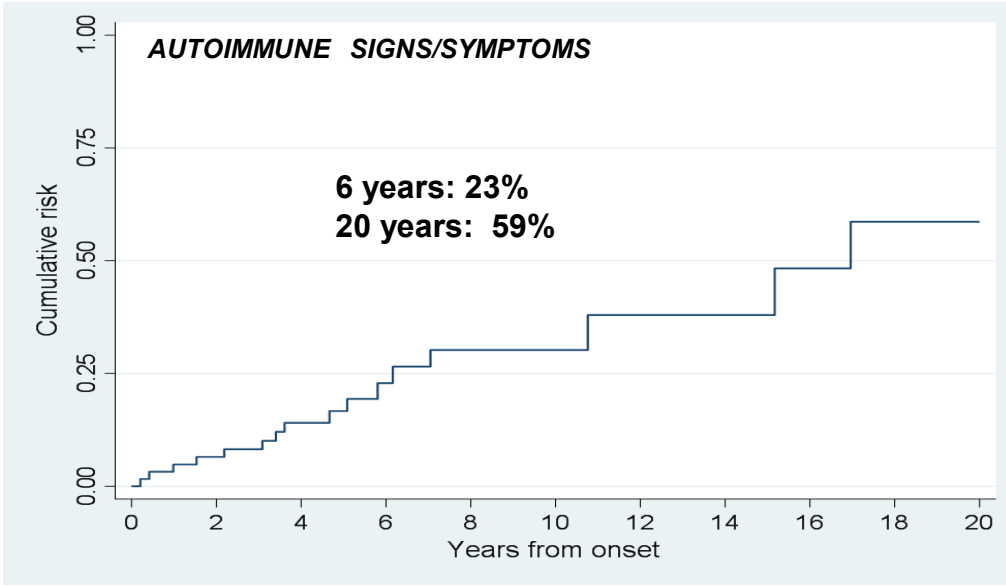
Low *B switched memory*

Increased *marginal zone B lymphocytes*

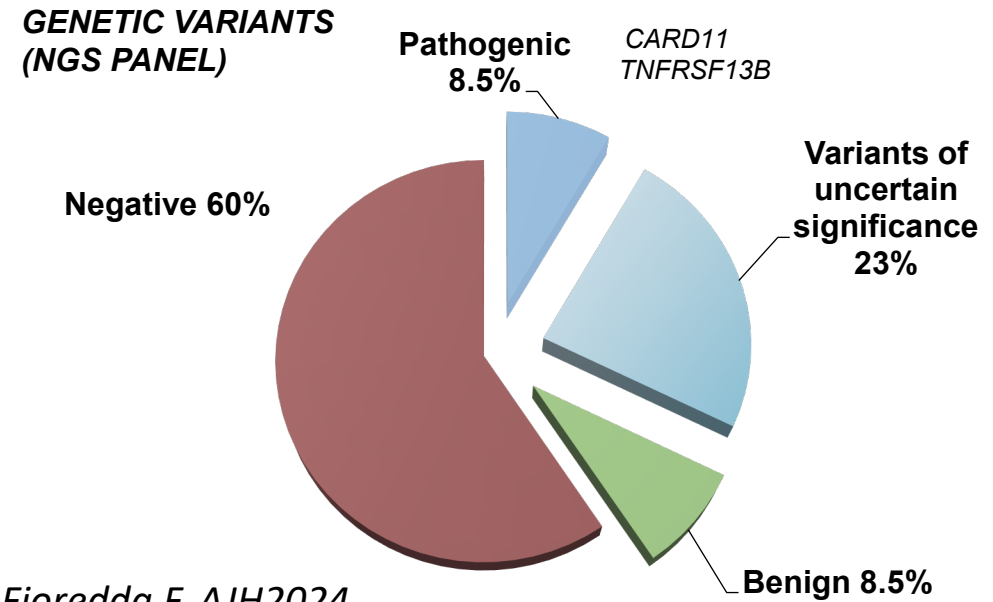
Increased *Double Negative B cells(total and DN2) and CD21 (DN1)*

Shift T *naive >>memory effector*

AUTOIMMUNE SIGNS/SYMPTOMS



GENETIC VARIANTS (NGS PANEL)



Fioredda F, AJH2024

FINAL CONSIDERATION AND DIAGNOSIS 2

CONGENITAL

- Isolated
- Associated with various extrahematological manifestations
- Associated with immunodeficiency/immune dysregulation
- Associated with metabolic disorders and nutritional deficiency
- Associated with bone marrow failure

ACQUIRED

- Primary or Idiopathic
  - ✓Antibody-mediated
  - ✓Non-antibody mediated
- Secondary due to
  - ✓Hypersplenism
  - ✓Infections
  - ✓Autoimmune diseases
  - ✓Nutritional deficiencies
  - ✓Hematologic diseases
  - ✓Drug-induced

LIKELY ACQUIRED

In children/adolescents, young adults

Late-onset/Long lasting

Fioredda F Haemasphere 2023

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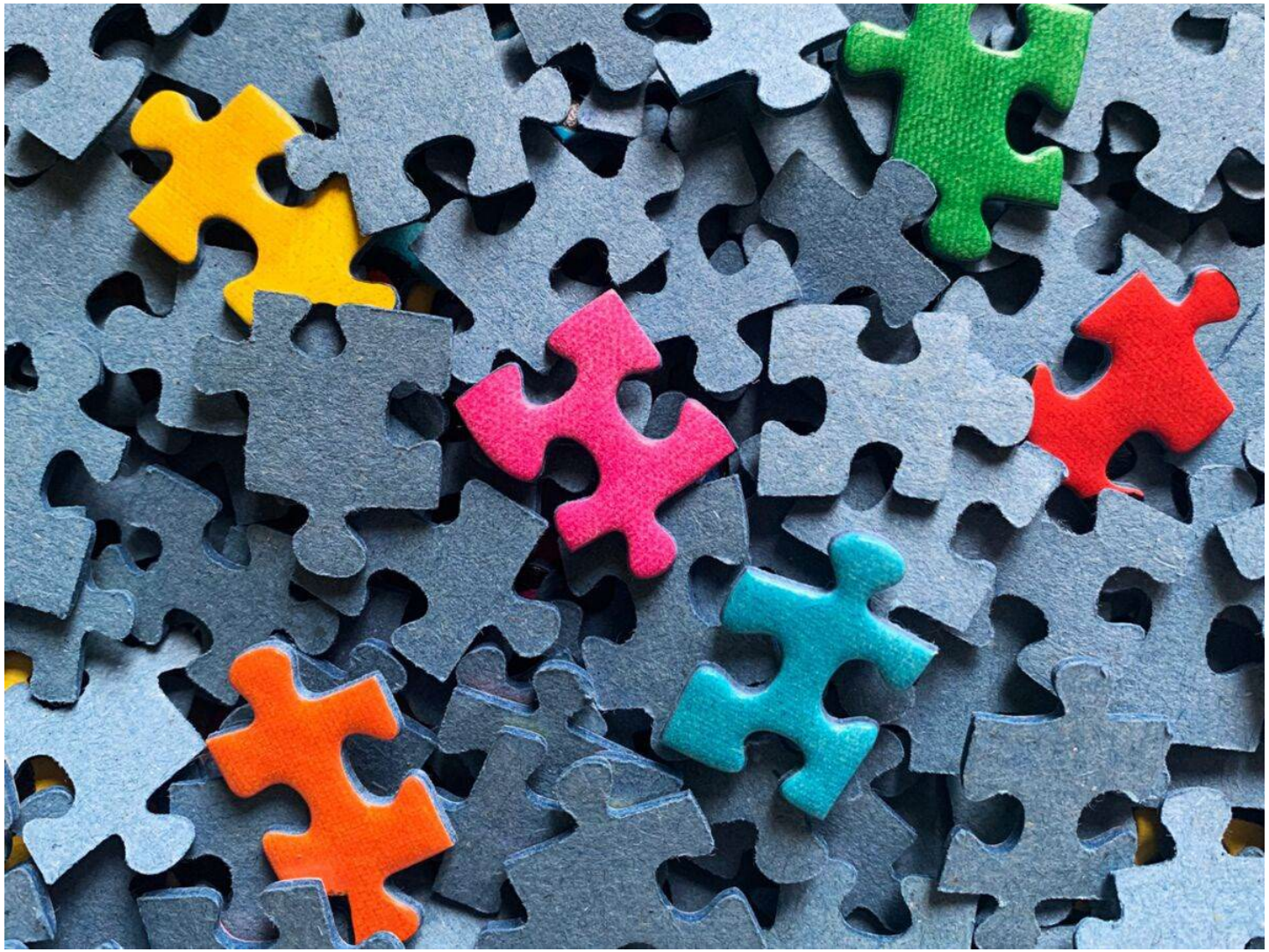
QUESTIONS



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Q1* **WHICH is the optimal combination of elements that together may have some relevance to orientate the diagnosis in this particular case?**

1 Age at onset, asthma, birth weight
2 Birth weight and no consanguinity
3 Ethnicity, gender, mild symptoms
4 Psoriasis, thyroiditis and age at onset
5 Psoriasis, gender and asthma

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Q2 * WHICH combination of blood tests would be more complete & informative to orientate the diagnosis in this case?

1) Lymphocyte subpopulations with double negative (DN), Autoimmunity, Antibody against Neutrophils
2) Antiphospholipid and anticardiolipin antibodies, flow cytometric analysis of large granular lymphocytes (LGL)/TCR clonality
3) Virology antibody screening and Ab against Neutrophils
4) Serum complement levels, next generation sequencing of gene panels related to myeloid malignancies to identify idiopathic cases at risk of development of MDS/AML
5) FBC in family members, serial blood counts twice a week over a period of 6 weeks to exclude cyclical neutropenia

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Q3 # In case of negativity of first sample for Ab against Neutrophils in a mildly symptomatic patient what is the suggestion according to the International Guidelines

1) To perform the MAIGA (monoclonal antibody immobilization of granulocyte antigens)
2) To look for anti-HLA antibodies against neutrophils
3) Repeat the I-GIFT two times
4) Repeat the I-GIFT several times
5) To perform bone marrow examination

Q4 # In the diagnostic phase of neutropenia, when is BM examination mandatory?

1) In all children affected with primary autoimmune neutropenia
2) In subjects affected with drug-induced neutropenia
3) In Ethnic Neutropenia/ADAN (ACKR1/DARC associated neutropenia)
4) In patients with severe and moderate/mild unexplained chronic neutropenia (any age)
5) In all children affected with primary Idiopathic neutropenia

Q5* Which elements in combinations may drive an ALPS diagnosis according to the NIH 2009 criteria?

1) Chronic benign lymphoproliferation, mild neutropenia, Ab against neutrophils, elevated DNT
2) FAS apoptosis test positive, elevated DNT, splenomegaly, block at promyelocyte stage in the bone marrow
3) Lymphoproliferation, Fas apoptosis test, neutropenia of any degree, family history of autoimmunity
4) Lymphoproliferation, elevated DNT, Fas Apoptosis test positive or ALPS-related gene, autoimmunity
5) Previous lymphoid malignancy, bilineage cytopenia, increased level of interleukin 2 (IL2), fever

Q6* What is the role of genetic testing in the present case?

1) To exclude the most common form of classical congenital neutropenia
2) To exclude cyclic neutropenia
3) To understand what is behind an unexplained long-lasting & late onset neutropenia
4) To study parents in view of a possible haemopoietic stem cell tranplantation (HSCT)
5) To be sure of not facing a post-infection neutropenia