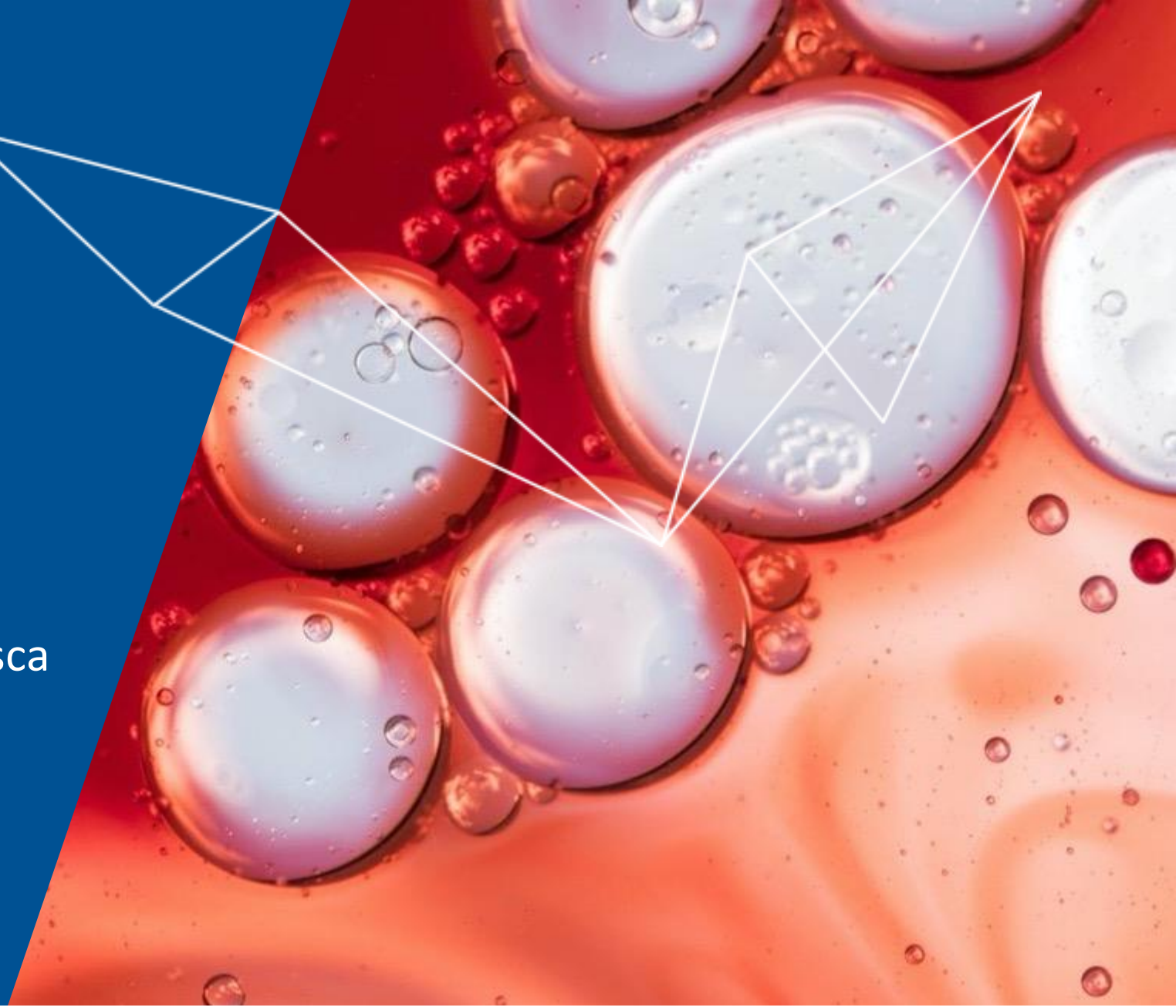
EUROPEAN HEMATOLOGY ASSOCIATION

EHA/INNOCHRON Guidelines on Chronic Neutropenias

Chairs: Helen Papadaki, Francesca Fioredda



European guidelines on diagnosis and management of neutropenia in adults and children

"The following recommendations have been agreed by the experts using a Delphi-like method and voted via a consensus-method. This is a preliminary draft of the EHA/EuNet-INNOCHRON guidelines on Chronic Neutropenias which has not been yet reviewed".

European guidelines on diagnosis and management of neutropenia in adults and children

Main Statements

Chapter 1: Definitions

Abbreviations

- SCN for severe chronic neutropenia
- CN for congenital neutropenia
- CyN for cyclic neutropenia
- AIN for autoimmune neutropenia
- IN for idiopathic neutropenia
- CIN for chronic idiopathic neutropenia
- ADAN for ACKR1/DARC associated neutropenia (previously called ethnic neutropenia)

✓Consensus reached 91% yes (12/12)

ACKR1/Duffy associated neutropenia (ADAN)

ACKR1/DARC-associated neutropenia (ADAN; previously called ethnic neutropenia, rs2814778 C/C in *ACKR1/DARC*) is defined as an ANC usually 0.5-1.5 x 109/L but occasionally below 0.5, mainly in populations originating from Africa or the Middle East.

✓Consensus: 77% YES, 15% NO, 8% not sure (13/13 votes)

1b. Classification of neutropenias

CONGENITAL			
	GENES INVOLVED	**TYPE OF INHERITANCE**	**MAIN FEATURES/NOTES**
ISOLATED			
Severe congenital neutropenia (SNC)	ELANE	AD	Arrest of N maturation in bone marrow
	CSF3R	AD	Arrest of N maturation in bone marrow Unresponsive to G-CSF
	CXCR2	AR	No arrest of maturation Myelokathexis
	WAS	X-linked	Maturation arrest Monocytopenia, lymphopenia
Cyclic neutropenia	ELANE	AD	Intermittent/cyclic impaired differentiation

	GENES INVOLVED	TYPE OF INHERITANCE	MAIN FEATURES/NOTES
ASSOCIATED TO:			
To various extra-hematological manifestations:			
Barth Sindrome (3-methylglutaconic aciduria type II)	TAZ	X-linked	No maturation arrest, Hypertrophic cardiomyopathy, Myopathic syndrome
Charcot-Marie-Tooth neuropathy type B	DNM2	AD	Distal limb muscle weakness and atrophy due to peripheral neuropathy
Cohen syndrome	VPS13B	AR	No maturation arrest, Psychomotor retardation, Microcephaly, Facial features, Hypotonia and joint laxity, Progressive retinochoroidal dystrophy, Myopia
G6PC3 mutation	G6PC3	AR	Skin hyper-elasticity and prominent superficial venous network, congenital heart disease, arrhythmias, uropathy, cryptorchidism, exocrine pancreatic insufficiency
GFi1 mutation	GFi1	AD	Sometimes maturation arrest/ Lymphopenia, increased numbers of immature myeloid cells in the peripheral blood Inner ear defect
HYOU1 mutation	HYOU1	AR	Hypoglicemia, inflammatory complications
JAGN1 mutation	JAGN1	AR	Sometimes maturation arrest, Bone and teeth abnormalities, exocrine pancreatic insufficiency
Kostmann disease	HAX1	AR	Maturation arrest, mental retardation/seizures, Susceptibility to MDS/AML
Neutropenia associated to SEC61A1 mutation	SEC61A1	AD	Maturation arrest
P14/LAMTOR2 mutation	LAMTOR2	AR	Chronic neutropenia Hypogammaglobulinemia No maturation arrest Oculocutaneous albinism, stunted growth

	GENES INVOLVED	TYPE OF INHERITANCE	MAIN FEATURES/NOTES
ASSOCIATED TO:			
To various extra-hematological manifestations: (continued)			
Pearson syndrome	Mitochondrial DNA deletions	Mitochondrial	Refractory sideroblastic anemia, Vacuolization of bone marrow precursors, Exocrine pancreatic dysfunction
Schimke immuno-osseus dysplasia	SMARCAL1	AR	Spondylo-epiphyseal dysplasia, Slowly progressive immune defect, Immune-complex nephritis
SMARCD2 mutation	SMARCD2	AR	Dysplastic syndrome, No granules in N Chronic diarrhea, bone abnormalities, low set ears
Specific granule mutation	CEBPE	AR	N with bilobed nuclei
TCIRG1 Neutropenia	TCIRG1	AD	Variable /No maturation arrest Skin angiomatosis
VPS45 mutation	VPS45	AR	myeloid hyperplasia/myelofibrosis,Nephromegaly, HSM, ,mental retardation, epilepsy, osteosclerosis
Wolcott-Rallison syndrome	EIF2AK	AR	Maturation arrest Insulin-dependent neonatal diabetes, epiphyseal dysplasia, growth retardation, hepatic and renal dysfunction, developmental delay, exocrine pancreatic deficiency

	GENES INVOLVED	TYPE OF INHERITANCE	MAIN FEATURES/NOTES
To immunodeficiency/immune-dysregulation			
Adenosine deaminase 2 deficiency	ADA2	AR	Severe combined immunodeficiency, Vasculitis, Cerebrovascular disease,Pure red cell aplasia, BMF
Autoimmune lymphoproliferative syndrome (ALPS)	FAS, FASLG, CASP10	AD	Lymphoproliferation, Autoimmune cytopenias
Cartilage-hair hypoplasia	RMRP	AR	Metaphyseal chondrodysplasia, Ligamentous laxity, Defective immunity Hypoplastic anemia, Intestine neuronal dysplasia
CD40L/Hyper IgM syndrome, type I	CD40L	X-linked	Severe infections,Autoimmune disease, Cancer predisposition
Chédiak-Higashi syndrome	LYST	AR	Decreased pigmentation of hair and eyes, Peroxidase-positive inclusion bodies in the myeloblasts and promyelocytes of the bone marrow,Peculiar malignant lymphoma
CLPB syndrome	CLPB	AD	Cataracts, Neurologic involvement
Familiar Haemofagocytic lymphohistiocytosis (FHLH)	PRF1, Perforin deficiency (FHL2)	AR	Fever, HSM, cytopenias
	UNC13D, UNC13D deficiency (FHL3)	AR	Fever, HSM, cytopenias
GATA2 syndrome	GATA2	AD	Monocytopenia , Deafness,HPV infections
Griscelli Syndrome, type II	RAB27A	AR	Hypomelanosis,Neurologic impairment
Hermansky-Pudlak syndrome type2	AP3B1	AR	Albinism
Reticular dysgenesis	AK2	AR	Severe Combined Immunodeficiency, Sensorineural deafness
STK4 mutation	STK4	AR	Intermittent NP, Monocytopenia, T- and B-lymphopenia, Atrial defect, HPV infections
WHIM syndrome	CXCR4	AD	No arrest of maturation, Myelokathexis, Lymphopenia, Cardiopathy (Tetralogy of Fallot)
Wiskott-Aldrich syndrome	WAS	X-linked	Eczema, Thrombocytopenia,Severe infections, Bloody diarrhea

	GENES INVOLVED	TYPE OF INHERITANCE	MAIN FEATURES/NOTES
To metabolic disorders and nutritional deficiency			
Gaucher Disease type I	GBA	AR	HSM, Thrombocytopenia, Osteolytic lesions
Glycogen storage disease Ib	SLC37A4/G6PT1	AR	Hepatomegaly, IBD, Fasting hypoglycemia
Isovaleric acidemia	IVD	AR	Neonatal ketoacidosis, Developmental delay, Lethargy, Feeding refusal
Methylmalonic acidemia	MMUT	AR	Lethargy, Failure to thrive, Recurrent vomiting, Hypotonia, Hepatomegaly ,Developmental delay
Propionic acidemia	PCCB, PCCA	AR	Lethargy, Cardiomyopathy, Feeding difficulties, Acute encephalopathy
Transcobalamin II deficiency	TCN2	AR	Developmental delay, Diarrhea/Vomit, Lethargy, Mucosal ulceration
To bone marrow failure			
Fanconi anemia	FANC complementation group	AR, X-linked (FANCB)	Congenital malformations,Cancer predisposition
Ribosomopathy Diamond –Blackfan Anemia Cartilage hair hypoplasia	RPS19, RPL5, RPS26, RPL11,..	AD	Congenital malformations ,Growth retardation, Urogenital tract and heart malformations
	GATA1	X-linked	Early-onset anemia, thrombocytopenia, bone marrow erythroid hypoplasia
	RMRP	AR	growth failure, immunodeficiency, cancer predisposition
	HEATR3, TSR2	AR, AD	Congenital malformations
SAMD9/SAMD9L syndromes	SAMD9/SAMD9L	AD	Adrenal insufficiency, Congenital malformations, Cerebellar ataxia, Severe invasive infections, MDS predisposition

	GENES INVOLVED	TYPE OF INHERITANCE	MAIN FEATURES/NOTES
To metabolic disorders and nutritional deficiency (Continued)			
SAMD9/SAMD9L syndromes	SAMD9/SAMD9L	AD	Adrenal insufficiency, Congenital malformations, Cerebellar ataxia, Severe invasive infections, MDS predisposition
Shwachman-Diamond syndrome	SBDS	AR	Mild neutropenia, Dysgranulopoiesis, mild dysmegakaryopoiesis, Exocrine pancreas deficienc, metaphyseal dysplasia, Mental retardation , Heart: cardiomyopathy
	EFL1	AR	Mild neutropenia, dyserythropoiesis, Exocrine pancreas deficiency, metaphyseal dysplasia, mental retardation
	DNAJC21	AR	Moderate neutropenia /mild dyserythropoiesis, Failure to thrive, developmental delay recurrent infections, hair/skin/teeth abnormalities
SRP54 mutation	SRP54	AD	Maturation arrest ,Exocrine pancreatic dysfunction
Telomers diseases	DKC1	X-linked	Mucocutaneous features Liver fibrosis Idiopathic pulmonary fibrosis Cancer predisposition
	hTR, TERT, TINF2, DKC1, ACD	AD	
	TERT, NHP2, NOP10, WRAP53, NOLA3,TCB1, RTEL1, CTC1, PARN	AR	
	USB1 (Clericuzio syndrome, poikiloderma with neutropenia)	AR	Retinopaty, developmenta delay, facial dysmorphisms, poikiloderma

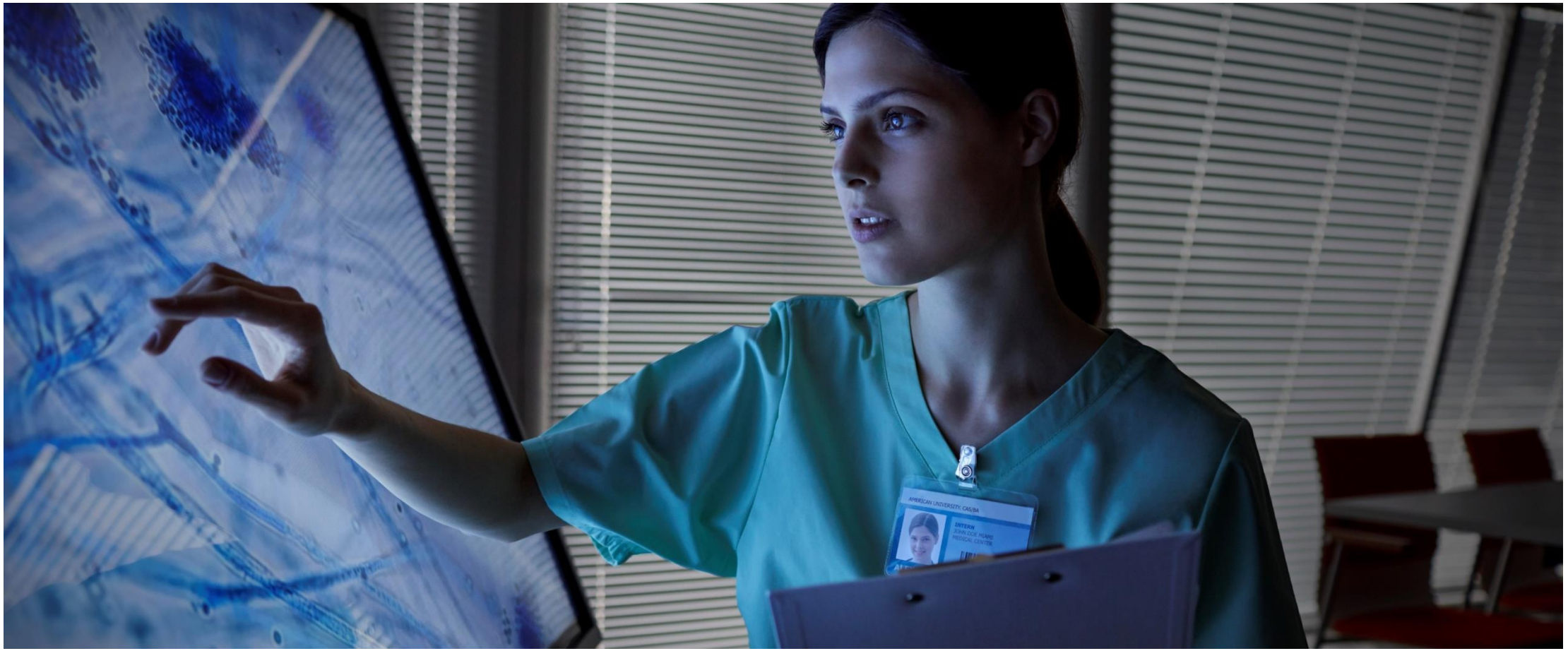
ACQUIRED	
PRIMARY OR IDIOPATHIC **Neutropenia as predominant , often isolated feature**	**Antibody-mediated** Primary autoimmune Primary alloimmune **Non Antibody mediated** Idiopathic Neutropenia of infancy Chronic Idiopathic Neutropenia (CIN)/Idiopathic Cytopenia of Undetermined Significance-Neutropenia (ICUS-N)
SECONDARY = **Neutropenia associated/due to**	**Hypersplenism** (Due to congestive, infiltrative, phagocytic, reactive splenomegaly) **Infections** Viral (e.g. HIV, HBV, HCV EBV, CMV), bacterial (e.g. Salmonella, Brucella, Rickettsia, Mycobacterium, Mycoplasma, H. Pylori), parasitic (e.g Plasmodium spp) , fungal (e.g. histoplasmosis) **Autoimmune Diseases** Organ specific e.g. Thyroid diseases, Inflammatory bowel disease, Primary biliary cirrhosis Systemic e.g. SLE, RA including Felty's Syndrome, Sjogren syndrome, Systemic sclerosis, Graft-vs-Host Disease **Nutritional Deficiencies** B12, folic acid, iron, copper, caloric malnutrition **Immuno-regulatory disorders** Common variable immunodeficiency, ALPS, ALPS-like diseases, Hemophagocytic Lymphohistiocystosis (HLH) Syndromes **Haemopathies** Primary benign e.g. aplastic anaemia Clonal (myeloid malignancies/lymphoid malignancies including LGL) **Drug-induced** Chemotherapy Non-chemotherapeutic drugs Analgesics and NSAIDs, Antibiotics (beta-lactams, cefipime, trimethoprim-sulfametoxazole, sulfasalazine, vancomycin, rifampicin, fluconazole, ketoconazole), Antidiuretics (, Furosemide, spironolactone), Antiretroviral (HIV) therapy, Antithyroids (tiamazofe, metimazole), Clozapine (olanzapine), Deferiprone, , Dipyrone (metamizole), Phenothiazines (alimemazine), Quinine/quinidine, IVIG, monoclonal antibodies (Rituximab), biological therapies (Infliximab, etanercept) Extended list of drugs associated with neutropenia can be found in PMID: 32531979, PMID: 29222255, PMID: 19459150, PMID: 17470834

Likely Acquired

In children /adolescents when neutropenia,both in the presence or not of the specific antibodies against neutrophils, arises or persists beyond age 5, the definition of late onset and long lasting neutropenia may be appropriate. Recent papers identified this «atypical neutropenia» as an epiphenomenon of immune dysregulation for typical biochemical/immunological features and the presence of variants in genes regulating immunity . The same is for several cases of aplastic anemia or ALPS/ ALPS-like syndrome, despite the age of occurrence.

✓ Consensus reached 92% yes, 8% unsure (12/12)

Chapter 2: Diagnostics



2.a What is important to know from patient/family history?

- Patients' history should include inquiry about occurrence of infections and their frequency, type and severity, and need for hospitalization. Specifically, history of omphalitis, gingivitis, periodontitis, skin infection abscess and pneumonias, as well as duration and response to antibiotics should be obtained. Presence of congenital malformations in the patient or family is also important

- For adult patients drug history is important, as well as, work-up for autoimmune and other disorders that may me associated with neutropenias

- Detailed family history should include ethnic origin, consanguinity, occurrence of recurrent infections and neutropenias in other family members, as well as unexplained infant death or miscarriages.

✓ Consensus reached 100% yes (13/13)

2b. What a detailed clinical examination should comprise (e.g. evidence of infection; lymphadenopathy and/or splenomegaly; congenital abnormalities associated with specific syndrome)?

- Careful clinical examination of skin and mucous membranes, upper and lower respiratory tract and abdomen to exclude underlying infection, lymphadenopathy and/or hepatosplenomegaly. Clinicians should be aware that neutropenic patients might have only subtle symptoms of infection due to reduced inflammatory response.

✓ Consensus reached 100% yes (13/13)

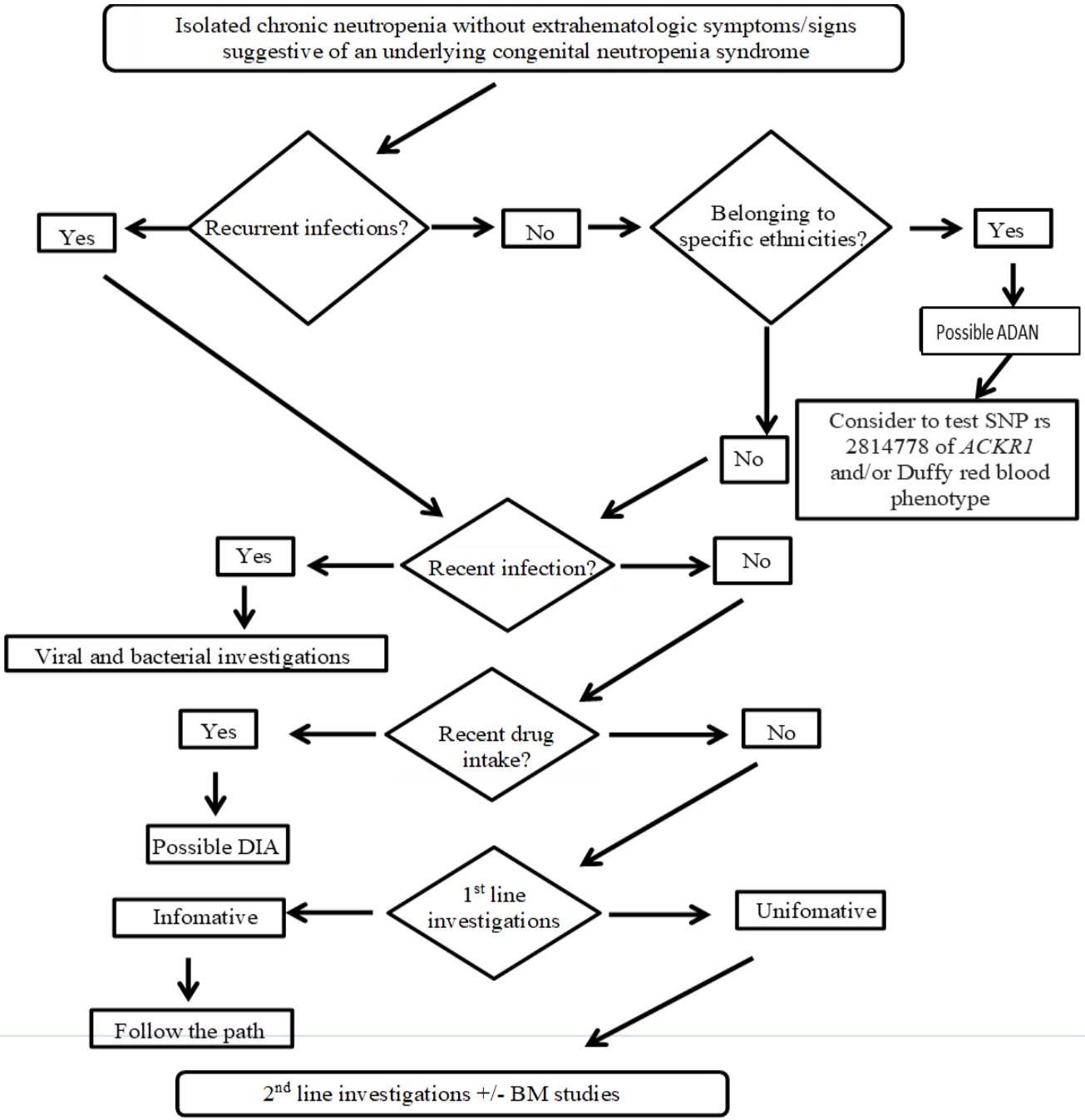
- In children and adults, clinical examination is crucial to detect congenital disorders. It should focus on growth, evidence of cognitive impairment, developmental delay, somatic dysmorphism (mainly skeletal), nail, hair or skin abnormalities, signs of bronchiectasis due to recurrent chest infections, hepatomegaly or splenomegaly, organ malformation, evidence of superficial veins and finally signs of photophobia, nystagmus, oculocutaneous albinism and neuropathy. The absence of obvious clinical signs does not exclude the presence of a congenital disorder.

✓ Consensus reached 91% yes 9% not sure (13/13)

- Cardiac function, presence of enlarged lymph nodes, joint symptoms and symptoms compatible with autoimmune, metabolic, gastrointestinal or nutritional diseases should also be considered.

✓ Consensus reached 91% yes 9% not sure (13/13)

2.c-d What tests should the basic and different levels of investigations include?



✓ Consensus: 100% YES (13/13 votes)

1st investigations in children (after referral to the specialist): complete blood counts; peripheral blood (PB) smear; biochemistry investigation including liver and kidney function;, C-reactive protein (CRP); vitamin B12 and folate; immunoglobulin serum level; Flow cytometric analysis of PB lymphocyte subsets; Virology Antibody screening: HepB, HepC, HIV, EBV, CMV, Parvovirus; indirect anti-neutrophil antibodies (GIFT, GAT, other); anti-thyroid antibodies (anti-TG, anti-TPO); evaluation of double negative CD3 lymphocytes in flow cytometric analysis of PB

1st investigations in adult (after referral to the specialist): complete blood counts; peripheral blood (PB) smear; biochemistry investigation including liver and kidney function;, C-reactive protein (CRP); vitamin B12 and folate; immunoglobulin serum level; Flow cytometric analysis of PB lymphocyte subsets; Virology Antibody screening: HepB, HepC, HIV, EBV, CMV, Parvovirus; indirect anti-neutrophil antibodies (GIFT, GAT, other); thyroid hormones (TSH, FT3, FT4); anti-thyroid antibodies (anti-TG, anti-TPO); anti-phospholipid, anti-cardiolipin antibodies; evaluation of LGL/TCR clonality in flow cytometric analysis of PB; serum ferritin; RF, ANA, ENA, ds-DNA; Erythrocyte Sedimentation Rate

2nd line investigations in children: evaluation of blood counts in family members; serial blood counts twice a week over a period of 6 weeks to exclude cyclic neutropenia; copper; ceruloplasmin; anti-gliadin antibodies; RF, ANA, ENA, ds-DNA

2nd line investigations in adults: evaluation of blood counts in family members; serial blood counts twice a week over a period of 6 weeks to exclude cyclic neutropenia; copper; ceruloplasmin; anti-gliadin antibodies; serum electrophoresis; serum complement factor/activation; next generation sequencing of gene panels related to myeloid malignancies to identify idiopathic cases at risk to MDS/AML development.

In children, young adults and considered for adults: genetic investigations.

*** BM examination includes aspiration, trephine biopsy, cytogenetics and flow cytometry. Detailed indications are described in the next section

STATEMENT. In case of isolated neutropenia without a phenotype suggestive of any specific disease/syndrome it is advisable to pursue the pathway described in the flow chart.

✓Consensus: 92% YES, 8% NO (13/13 votes)

2.e. What is the role of BM examination? When? How often?

A diagnostic bone marrow with morphology, cytogenetics and myeloid NGS should be performed

1) In pediatric patients with severe and moderate chronic neutropenia with the exception of patients with primary autoimmune neutropenia with positive anti-granulocyte antibodies and drug-induced neutropenias.

2) In patients with suggested AIN but negative granulocyte antibody test, if patients suffer from recurrent infections.

3) In any patients prior to G-CSF treatment.

4) In all adult patients with unexplained chronic neutropenia with the exception of those with long-standing, mild, isolated neutropenia that remains stable over time.

✓ Consensus reached 82% yes (13/13)

Annual bone marrow and cytogenetics follow-up should be performed in patients

1) With congenital bone marrow failure syndromes independent of ANC and treatment with G-CSF

2) With undefined severe neutropenia (after extensive investigation) with G-CSF treatment, may be considered

✓ Consensus reached 91% yes (13/13)

Repeated bone marrow follow-up should be performed in patients

With decreasing neutrophil counts or additional changes in other blood cell counts (e.g. anemia, thrombocytopenia) or erythrocyte indices

✓ Consensus reached 100% yes (13/13)

2.f. What is the role of anti-neutrophil antibody testing? When? Patient selection? What are the recommended tests? How should we interpret positive results?

- As stated in part 2c-d, antineutrophil antibody testing should be performed as first line investigation in both children and adults.
- Indirect GIFT is recommended as a first line assay in reference laboratories
- A positive GIFT in combination with laboratory tests and clinical picture can support diagnosis of AIN but does not exclude diagnosis other types of neutropenia
- With a negative indirect GIFT, if the clinical suspicion of AIN remains high, GIFT should be repeated several times.

✓Consensus reached 100% yes (13/13)

2.g-h. What is the role of genetic testing, which genes and method and what position in algorithm

- Genetic diagnosis is important to confirm the diagnosis of CN, estimating the risk for MDS/AML, supporting stem cell donor selection for patients and family counselling.
- When the clinical picture, inheritance or bone marrow features (ie block at the promyelocyte stage) are indicative of a specific gene mutation, single gene analysis by Sanger sequencing technique could be applied.
- For congenital neutropenia where the clinical picture does not suggest a specific genetic cause, we recommend using next generation sequencing (NGS) techniques such as multigene panels or targeted whole exome sequencing (WES).
- For patients for whom a genetic cause is not identified by the above methods, whole genome sequencing (WGS) and RNA-seq may provide powerful diagnostic tools.
- NGS analysis of bone marrow or peripheral blood for acquired somatic variants is recommended for patients with chronic unexplained neutropenia.
- Screening for known mutations is recommended in family members .
- Important to validate germline mutations in fibroblasts or hair follicles in some cases.

✓ Consensus reached 91% yes (13/13)

2i. The role of flow cytometry (FC) for the diagnosis of CNP

- FC of peripheral blood lymphocytes and BM granulocytic cells should be included in the diagnostic algorithm of adult patients with chronic neutropenias to support LGL leukemia and MDS diagnosis, respectively.
- FC is an important tool in the diagnosis of neutropenia associated PID syndromes such as ALPS, CVID, HIGM.
- Assessment of a PNH clone by flow cytometry testing is recommended.

✓ Consensus reached 82% yes (13/13)

Chapter 4: Natural history

4. a-c-d. Which groups of patients need a follow-up (CBC, BM, Cytogenetics, NGS) ? How often?

- As stated in part 2e, annual BM and cytogenetics and myeloid NGS should be performed in all patients with congenital BM failure syndromes independent of ANC and treatment with G-CSF to assess clonal hematopoiesis and for early diagnosis of MDS or leukemia.
- In chronic neutropenia patients, it is recommended to perform CBC with differential white blood cell counts and morphological evaluation every 3-4 months.
- When approaching adulthood CN patients should be transferred to a dedicated hematology specialist.

✓ Consensus 100% (12/12 votes)

4. a-c-d. Which groups of patients need a follow-up (CBC, BM, Cytogenetics, NGS) ? How often?

Adults

- As already stated in part 2e, a diagnostic BM with morphology and cytogenetics should be performed in all adult patients with unexplained chronic neutropenia with the exception of those with long-standing, mild, isolated neutropenia that remains stable over time.
- As already stated in part 2g, NGS analysis of BM or peripheral blood for acquired somatic variants is recommended for patients with chronic unexplained neutropenia.
- Patients with mutations in one or more MDS/leukemia associated genes, particularly with high VAF (>10%) and especially younger patients where the frequency of CHIP is low, need to have a closer follow-up (more than 4 times a year) with CBC, differential white blood cell count and morphological examination. BM examination including aspiration, trephine biopsy, karyotype and NGS analysis should be performed upon indication i.e. worsening of cytopenias, macrocytosis, and/or morphological abnormalities.

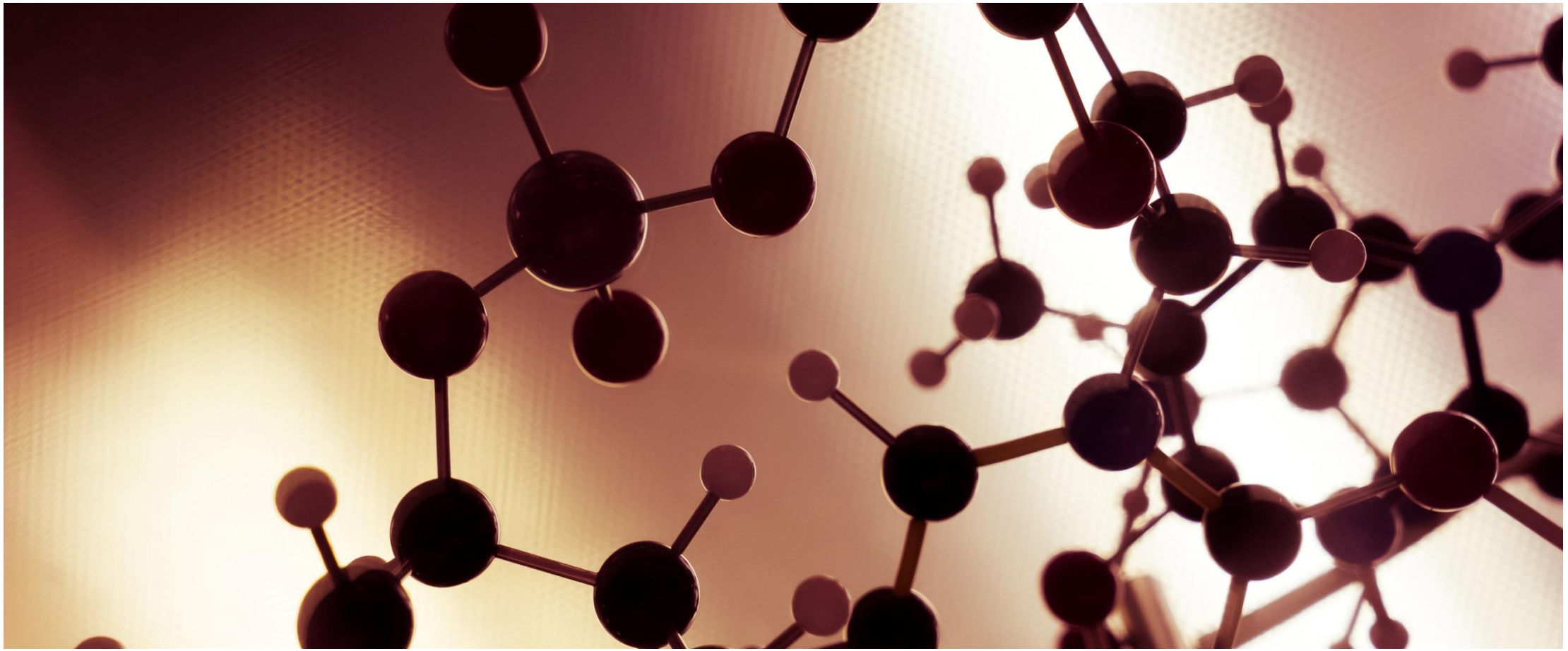
✓ Consensus 83% yes, 17% not sure (12/12 votes)

4.b. Are there any prognostic indicators of transformation into MDS/Leukemia?

- Typical morphology for MDS in peripheral blood and/or bone marrow, cytogenetic abnormalities (trisomy 21, and monosomy 7), somatic leukemia-associated mutations (e.g. CSF3R, RUNX1 and ASXL1) and multi-hit TP53 mutations in SDS are prognostic indicators of development into MDS/AML

✓ Consensus: 100% YES (12/12 votes)

Chapter 5: Special situations



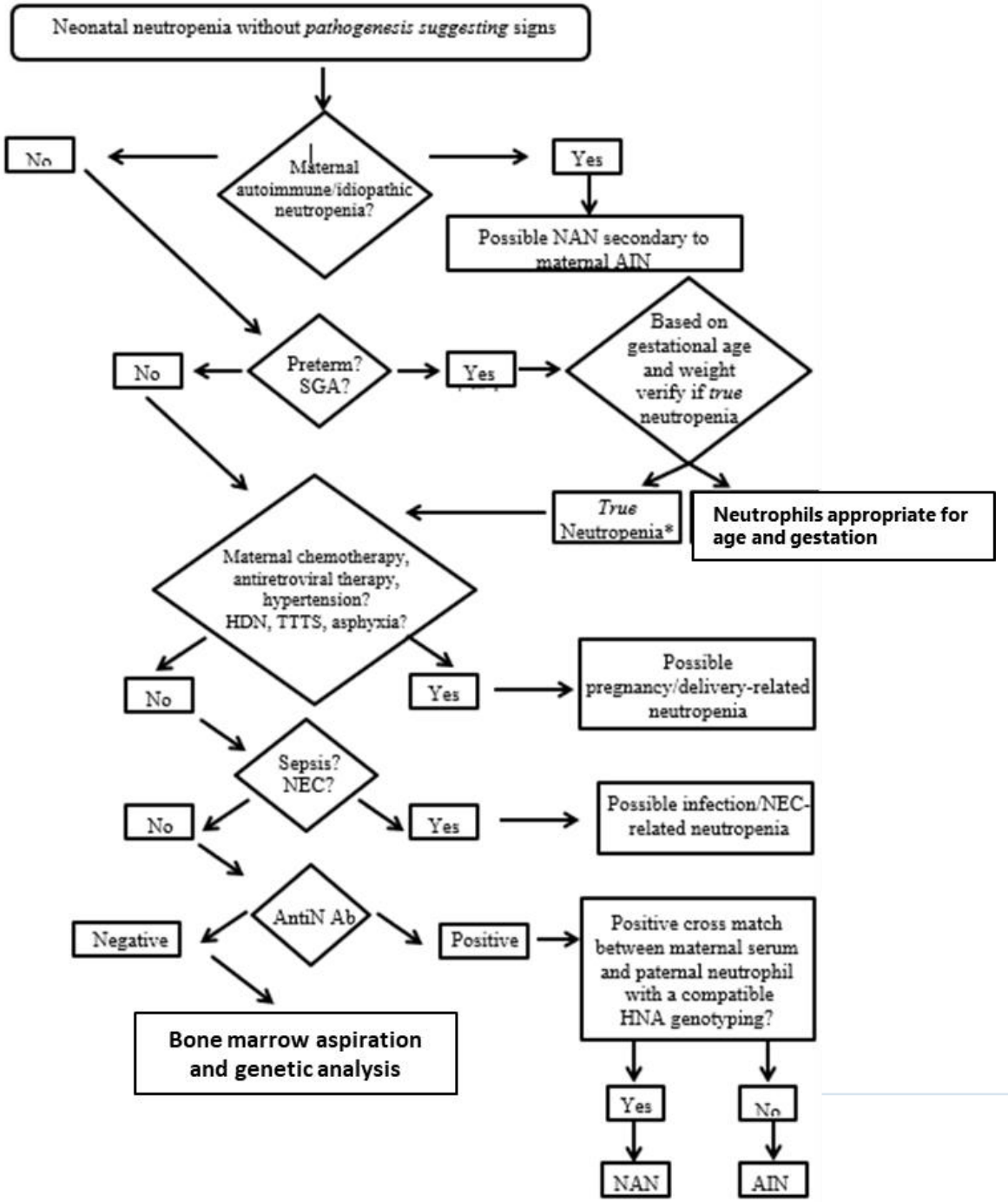
5.a. Pregnancy

- The panel recommends G-CSF treatment to all patients who were on G-CSF treatment before pregnancy and all patients with severe congenital neutropenia.
- For patients who were not on treatment, G-CSF treatment might be considered.
- It also suggested to frequently evaluate ANCs during G-CSF therapy in pregnancy, particularly in patients with autoimmune neutropenia, because a physiological increase can be seen.
- After delivery, the neutrophil count of the newborn should be checked.

✓Consensus 100% yes (13/13)

5.b. Neonates

- In case of isolated neutropenia without a phenotype suggestive of any specific disease/syndrome it is advisable to pursue the pathway described in the flow chart of the Figure.



NAN: Neonatal Alloimmune Neutropenia; AIN: Autoimmune Neutropenia; SGA: Small for Gestational Age; HDN: Rh-Hemolytic Disease of the Newborn; TTTS: Twin-Twin Transfusion Syndrome; NEC: Necrotising Enterocolitis; AntiN: Anti-Neutrophil; Ab: antibodies; HNA: Human Neutrophil Antigen; *The neutropenia is defined as "true" if the ANC is out of the range of the corresponding Gestational Age and there is not a prenatal growth retardation

5.c. COVID-19 and response to vaccines

- All patients with chronic neutropenia should take up the offer of approved vaccines

✓ Consensus: 90% yes, 10% no (13/13)

5.d. G-CSF administration in patients with COVID-19 disease

- Based on reports of patients under chemotherapy or immunosuppression with neutropenia and COVID-19 disease who displayed a respiratory failure following G-CSF, and despite the lack of a clear causal association, clinicians should be aware of this potential side effect when administering G-CSF in neutropenia patients with COVID-19.