IN The Name Of God

Congenital Neutropenia

Presenting by
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Out lines

- Identify in the clinical presentation of a patient with neutropenia **the key features suggesting a possible genetic cause**
- Realize that in G6PC3 neutropenia and in GSDIB, gliflozine may be considered an alternative to standard care with GCSF and/or HSCT
- Learn that in CXCR4 WHIM syndrome, CXCR4 inhibitors offer a therapeutic approach

Introduction

- Functional neutrophils are the most subpopulation of human leukocytes, representing the first line of the innate immune system defense

- They exhibit an advanced antimicrobial mechanisms, from which we distinguish phagocytosis with, oxidative burst, release of nuclear material in the neutrophil extracellular traps (NETs) or degranulation of neutrophil granules

- The main factor regulating both proliferation of neutrophil precursors and mature neutrophils release form the bone marrow is granulocyte colony-stimulating factor

Introduction

- Disturbed production of neutrophils during hematopoiesis results in neutropenia in the peripheral blood, which leads to immunodeficiency.
- Among many causes of neutropenia, numerous genetic defects are described causative for congenital neutropenia Mutations of neutrophil **elastase gene (ELANE)** are ones of the most commonly observed in patients suffering from congenital neutropenia.
- Nowadays, pathogenesis of these defects is still arguable and controversial, thus, it attracts a great scientific focus.

Diagnosis and main features of congenital neutropenia

- Neutropenia is reduction in the absolute number of neutrophils circulating in the blood below 1500
- profound when 500 chronic if it lasts more than 3 months (intermittent or permanent).
- The exception is the first weeks of life, during which the number of neutrophils is physiologically elevated.

Congenital neutropenia

- Congenital neutropenia (CN) is a family of genetic diseases associated with three main features:
- low neutrophil count
- susceptibility to infection, various organ dysfunctions
- high risk of leukaemic transformation.

Congenital neutropenia: an evolving definition

- The discovery of the genes responsible for different subtypes of CN induced a change in the disease classification.

- The term ‘congenital neutropenia’ is not homogeneously used in the literature
- Aim to not to restrict the definition of CN to disorders in which neutropenia is the only phenotypic manifestation but have considered all genetic disorders comprising neutropenia as a chronic or recurrent manifestation .

ELANE MUTATIONS IN PATHOGENESIS OF CONGENITAL NEUTROPENIA

- Heterozygous mutations identified in ELANE gene result in a SCN, which might be a life threatening condition, or a cyclic neutropenia (CyN) with moderate to mild clinical features.
- Pathogenesis of SCN and CN is a subject of many studies and scientists are still not able to unequivocally explain how dysfunctions in NE lead to maturation arrest of granulocytic differentiation.
- The role of NE defects in myelocytes maturation arrest in bone marrow is widely investigated; however, the mechanism underlying this phenomenon has still remained unclear.

Table I. List and main features of known genes in congenital neutropenia (as of May 2017).

Subgroup of neutropenia	Gene/disease name (reference)	OMIM code	Main haematological features	Extra-haematopoietic features	Inheritance Gene localization	Normal function of the gene
CN usually without extra-haematopoietic manifestations	*ELANE* Severe congenital neutropenia/cyclic neutropenia (Horwitz *et al*, 1999; Dale *et al*, 2000)	202700 162800	Severe and permanent Maturation arrest Intermittent/cyclic with variable bone marrow features	No	Dominant 19q13.3	Protease activity Antagonism with alpha 1 antitrypsin
	CSF3R/Germline mutation of CSF3R (Triot *et al*, 2014)	202700	Permanent Maturation arrest Unresponsive to G-CSF	No	Dominant 1p35-p34.3	Transmembrane G-CSF receptor/intracellular signalling
	WAS/Severe congenital neutropenia (Devriendt *et al*, 2001)	301000	Severe and permanent Maturation arrest Monocytopenia	No	X Linked Xp11.4-p11.21	Cytoskeleton homeostasis
	CXCR2/chronic neutropenia (Auer *et al*, 2014)		Severe and permanent No maturation arrest Myelokathexis	No	Recessive 2q35	Chemokine receptor (CXCL12)
CN with frequent extra-haematopoietic manifestations, including innate immunity deficiencies	*SBDS*/Shwachman-Bodian-Diamond disease (Boocock *et al*, 2003)	260400	Mild neutropenia Dysgranulopeosis, mild dysmegakaryopoiesis	Exocrine pancreas deficiency, bone: metaphyseal dysplasia, mental retardation, heart: cardiomyopathy	Recessive 7q11.22	Ribosomal protein Regulation of RNA expression
	EFL1 syndrome (Stepensky *et al*, 2017)	260400	Mild neutropenia dyserythropoeisis	Exocrine pancreas deficiency, bone: metaphyseal dysplasia, mental retardation,	Recessive 15q25.2	Ribosomal protein Regulation of RNA expression
	GATA2 syndrome (Collin *et al*, 2015)	614038 614172	Mild neutropenia, monocytopenia Macrocytosis	Lymphedema, deafness, mycobacteria HPV infections	Dominant 3q21.3	Transcription factor
	G6PC3/Severe congenital neutropenia (Boztug *et al*, 2009)	202700	Maturation arrest	Skin-prominent superficial venous network, heart: atrial defect, uropathy	Recessive 17q21	Glucose 6 – phosphatase complex catalytic unit
	SLC37A4/Glycogen storage type Ib (Veiga-da-Cunha *et al*, 1999)	232220	No maturation arrest	Hypoglycaemia, fasting hyperlactacidaemia and glycogen overload of the liver	Recessive 11q23.3	Glucose 6 – phosphatase complex trans ER transporter
	TAZ/Barth disease (Barth *et al*, 1999)	302060	No maturation arrest	Hypertrophic cardiomyopathy, myopathic syndrome, 3 methyl glucaconic aciduria	X Linked Xq28	Tafazzin, phospholipid membrane homeostasis
	CXCR4/WHIM syndrome (Gorlin *et al*, 2000)	193670	Severe and permanent No maturation arrest Myelokathexis	Lymphopenia, thrombocytopenia Cardiopathy type, Tetralogy of Fallot	Dominant 2q21	Chemokine receptor (ligand CXCL12)
	JAGN1/Severe congenital neutropenia (Boztug *et al*, 2014)	616022	Variable	Bone abnormalities, exocrine pancreatic enzyme	Récessif 3p25.3	ER protein

Congenital neutropenia: the best strategy for finding germline mutations

The integration of NGS technologies into diagnostic practice allows the simultaneous analysis of multiple genes in a single assay at a similar cost to testing a few genes by Sanger sequencing.

Targeted NGS panels, including the protein coding regions and conserved splice sites of the known genes involved in isolated or syndromic CN have been developed for the diagnosis of CN.

The design of these panels can easily and regularly be updated by integrating the genes most recently reported in literature.

Congenital neutropenia: the best strategy for finding germline mutations

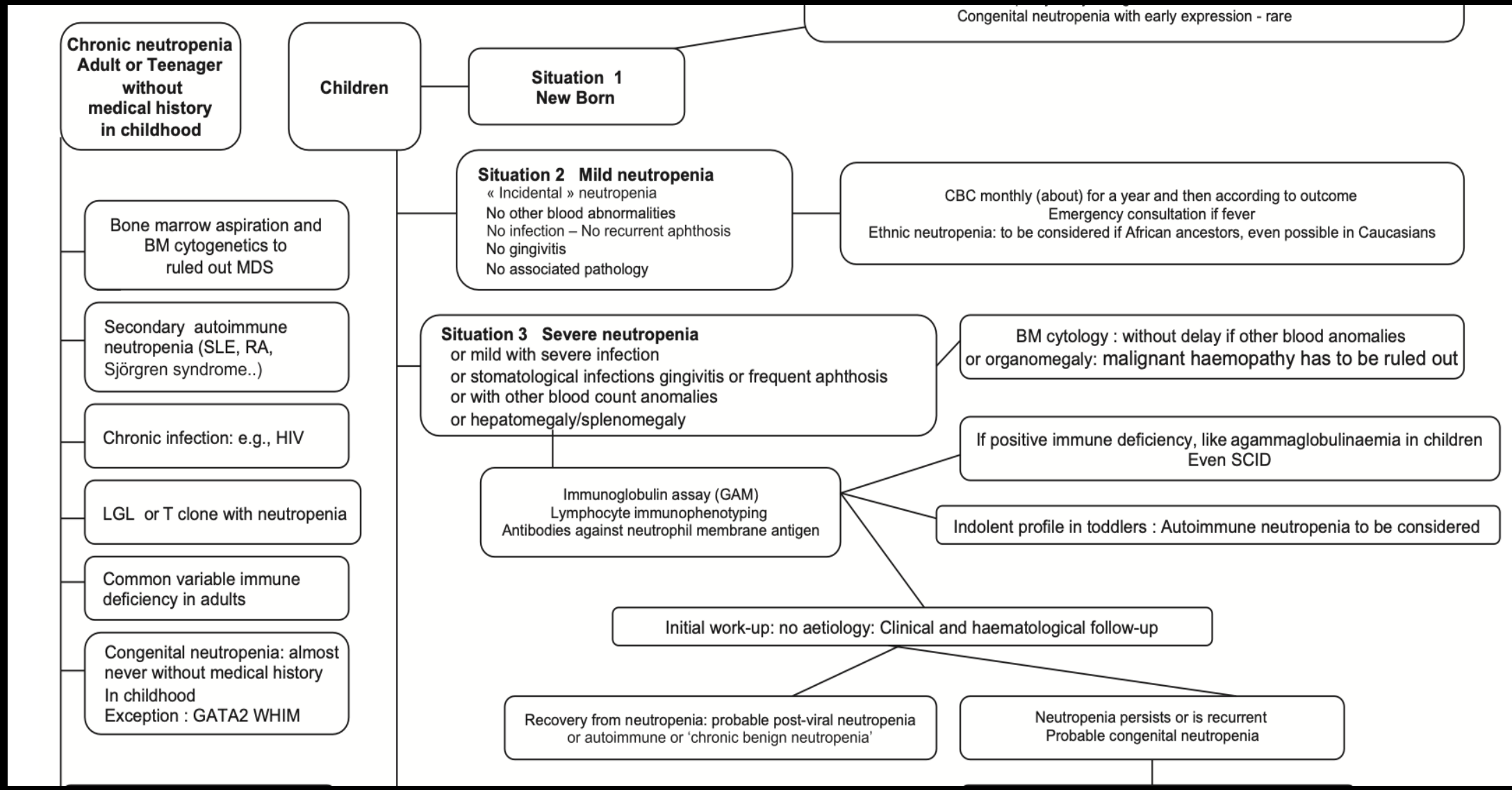
After analysis based on targeted NGS, about 40% of CN remain without molecular aetiology

Whole exome sequencing (WES) is a powerful strategy for the identification of novel genetic aetiologies, as shown by several groups that discovered novel causative genes in rare forms of syndromic CN such as VPS45, CLPB, EFL1, SMARCD2

In addition, the search for pathogenic copy-number vari- ants or for regions of homozygosity in case of consan guineous individuals should be considered.

When to consider congenital neutropenia as a possible diagnosis and how to confirm it?

- the initial interview and physical examination and the careful reading of the complete blood count are sufficient.
- The collection of information, such as consanguinity, geographic origin and familial history of neutropenia is important.
- Bone marrow examination is not mandatory for the diagnosis of all neutropenia, but should be performed to rule out malignant haemopathies


Chronic neutropenia
Adult or Teenager
without
medical history
in childhood
Children
Situation 1
New Born
Congenital neutropenia with early expression - rare
Situation 2 Mild neutropenia
« Incidental » neutropenia
No other blood abnormalities
No infection – No recurrent aphthosis
No gingivitis
No associated pathology
CBC monthly (about) for a year and then according to outcome
Emergency consultation if fever
Ethnic neutropenia: to be considered if African ancestors, even possible in Caucasians
Bone marrow aspiration and
BM cytogenetics to
ruled out MDS
Secondary autoimmune
neutropenia (SLE, RA,
Sjörgren syndrome..)
Chronic infection: e.g., HIV
LGL or T clone with neutropenia
Common variable immune
deficiency in adults
Congenital neutropenia: almost
never without medical history
In childhood
Exception : GATA2 WHIM
Situation 3 Severe neutropenia
or mild with severe infection
or stomatological infections gingivitis or frequent aphthosis
or with other blood count anomalies
or hepatomegaly/splenomegaly
BM cytology : without delay if other blood anomalies
or organomegaly: malignant haemopathy has to be ruled out
Immunoglobulin assay (GAM)
Lymphocyte immunophenotyping
Antibodies against neutrophil membrane antigen
If positive immune deficiency, like agammaglobulinaemia in children
Even SCID
Indolent profile in toddlers : Autoimmune neutropenia to be considered
Initial work-up: no aetiology: Clinical and haematological follow-up
Recovery from neutropenia: probable post-viral neutropenia
or autoimmune or 'chronic benign neutropenia'
Neutropenia persists or is recurrent
Probable congenital neutropenia

Bone marrow cytology

- Myelokathexis, defined by an increase in the granulocyte pool, with hypermature dystrophic neutrophils, indicating WHIM syndrome
- Condensed chromatin and hyposegmented neutrophils are in favour of Shwachman-Diamond syndrome what- ever the nutritional status
- Haemophagocytosis of neutrophils is a sign of autoim mune neutropenia in young children associated with a normal myeloid maturation
- Abnormal cytoplasmic granulations are suggestive of Chediak Higashi disease

A common consequence of neutropenia: infections

Whatever the underlying causes of neutropenia, a central consequence of the phenotype of CN is severe infections.

The most determinant factor linked with infection risk is the **residual capacity to mobilize neutrophils from the site of production to the site of infection**

In typical CN with profound neutropenia, the risk is not as severe as in drug- induced neutropenia and the risk of fungal infections is very low, far lower than in other phagocyte disorders, such as chronic granulomatosis disease.

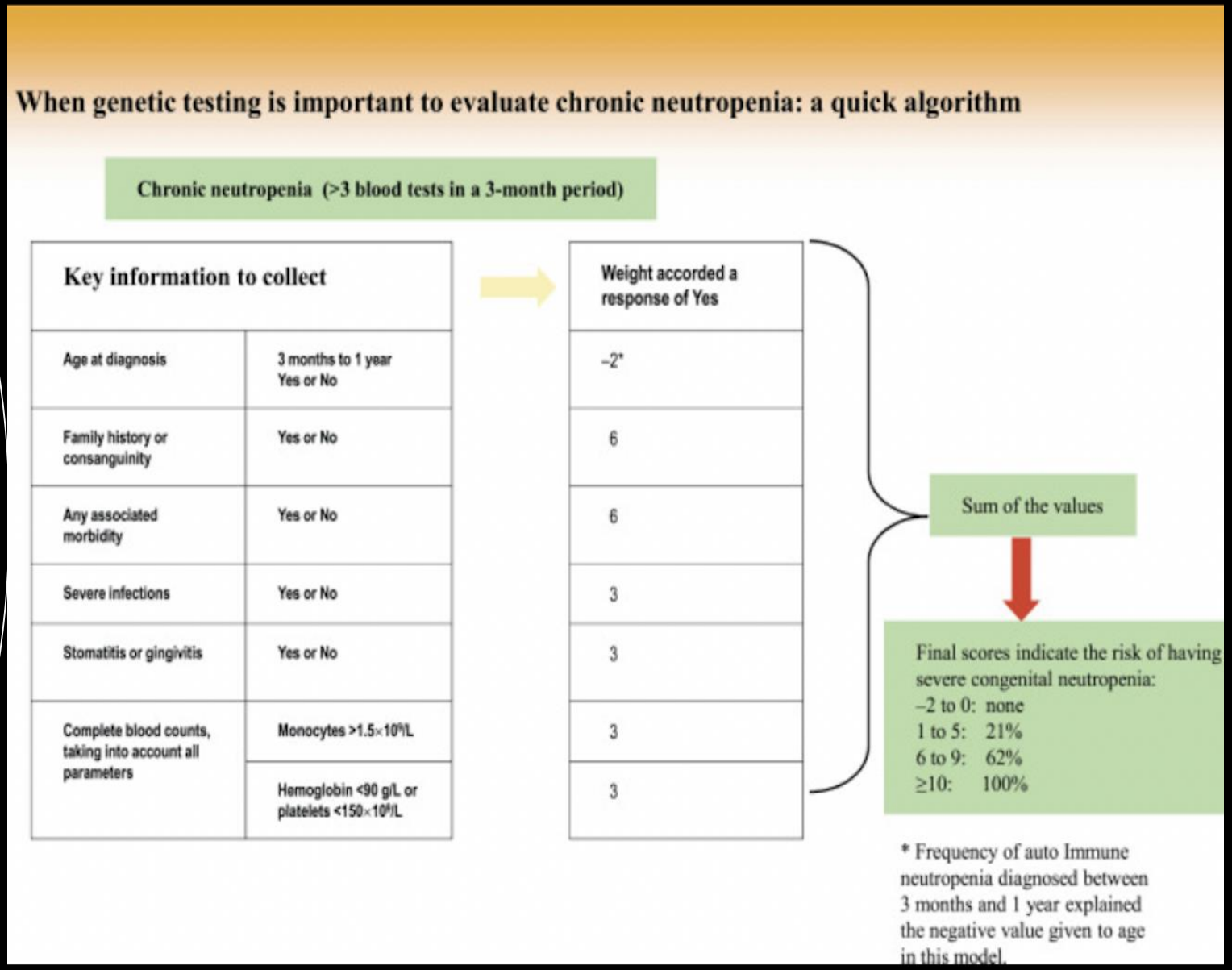
Infections

- The sites of infections are variable, skin and mucosa; the ear, nose, and throat; and the lungs.
- Stomatological infections are frequent after 2 years of age in patients with profound cneutropenia, and are characterized by erosive, haemorrhagic, and painful gingivitis associated with papules
- Two specific opportunistic infections may be found in a limited group of disorders belonging to this family; human papilloma virus (HPV) infections are very frequent in WHIM syndrome, GATA2 syndrome and STK4 deficiency, as well as in tuberculosis and atypical mycobacterial infections.

SCN genetics: accurate and quick diagnoses for better classification and more therapeutic options

- Some entities may share symptoms attributable to different genes, while very different clinical manifestations can be caused by the same genetic mutation(s).
- the term “SCN” is not completely accurate, and calling these entities genetic neutropenias would be more appropriate.
- Notably, genetic neutropenias (GNs) are not always severe, and the adjective “severe” is not justified by many patients’ clinical status.
- Now, obviously, genetic expertise is the key to diagnosis. Although blood tests have become routine examinations.

- A rapid decision of whether or not to launch genetic research can then be made by calculating the score of the individual algorithm elements



Management of congenital neutropenias

'Classic' management of congenital neutropenias

1 Evaluation of the individual's infectious susceptibility

2 Routine follow-up with sequential blood tests

3 Management of associated comorbidities

4 Evaluation of leukemic/myelodysplastic features

Main intervention

GCSF at adapted dose and schedule

HSCT

Genetics-driven management of congenital neutropenias

According to the genetic cause

- Poorly known mechanisms → Still 'classic' management
- Specific mechanisms → Adapted management

Gene and/or disease	*G6PC3* GSDIb	*CXCR4* WHIM syndrome	*SBDS* Shwachman–Diamond syndrome
Key hematological & infectious features	Susceptibility to bacterial & oral infections Inflammatory bowel disease	Susceptibility to bacterial & Human Papilloma virus infections	Low susceptibility to bacterial infection High risk of leukemia
Main pathophysiological features	1,5-Anhydro-D-glucitol high level	CXCR4 over activation	Clonal evolution with secondary TP53 and EIF6 clones. EIF6 inhibition represents a somatic

But once a genetic diagnosis is fully determined, how can this information change the natural history of a patient’s disease?

- The standard care of GNs is based on GCSF and HSCT.
- Driven by the extensive development of genetic background analyses of such entities, better understanding of the mechanisms at work now offers some possibilities of adapted therapy.
- ISGTL2, an antidiabetic drug, may partially reverse GSDIB and G6PC3 GNs by clearing 1,5AG, which is responsible for the associated neutropenia.
- CXCR4 inhibitors contribute to reversing the leukocyte defect in WHIM syndrome.
- Concerning the leukemic transformation risk, better understanding of the clonal evolution raises the possibility of preventing leukemia by **stimulating somatic genetic rescue,** a physiological process that might limit the risk of such progression.

Take Home Message

- Treatment of severe chronic neutropenia should focus on prevention of infections, the management of associated organ dysfunction and the prevention of leukaemic transformation
- . Such risks may be monitored by targeted NGS, which should detect somatic mutations involved in myeloid leukaemia or myelodysplasia.

- Granulocyte colony-stimulating factor and hematopoietic stem cell transplantation are now the bedrock of standard care.
- Better understanding of SCN mechanisms now offers the possibility of adapted therapy for some entities. An inhibitor of sodium glucose cotransporter, an antidiabetic drug, may attenuate glycogen storage disease type Ib and glucose-6-phosphatase catalytic subunit 3 neutropenias by clearing 1,5-anhydroglucitol, the precursor of the phosphate ester responsible for these SCNs.

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