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Cytogenetics in the management of bone marrow failure syndromes: Guidelines from the Groupe Francophone de Cytogénétique Hématologique (GFCH)

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ABSTRACT

Bone marrow failure syndromes are rare disorders characterized by bone marrow hypocellularity and resultant peripheral cytopenias. The most frequent form is acquired, so-called aplastic anemia or idiopathic aplastic anemia, an auto-immune disorder frequently associated with paroxysmal nocturnal hemoglobinuria, whereas inherited bone marrow failure syndromes are related to pathogenic germline variants. Among newly identified germline variants, *GATA2* deficiency and *SAMD9/9L* syndromes have a special significance. Other germline variants impacting biological processes, such as DNA repair, telomere biology, and ribosome biogenesis, may cause major syndromes including Fanconi anemia, dyskeratosis congenita, Diamond-Blackfan anemia, and Shwachman-Diamond syndrome. Bone marrow failure syndromes are at risk of secondary progression towards myeloid neoplasms in the form of myelodysplastic neoplasms or acute myeloid leukemia. Acquired clonal cytogenetic abnormalities may be present before or at the onset of progression; some have prognostic value and/or represent somatic rescue mechanisms in inherited syndromes. On the other hand, the differential diagnosis between aplastic anemia and hypoplastic myelodysplastic neoplasm remains challenging. Here we discuss the value of cytogenetic abnormalities in bone marrow failure syndromes and propose recommendations for cytogenetic diagnosis and follow-up.

1. Introduction

Bone marrow failure syndromes (BMFS) are rare hematological disorders with an incidence of 2-3 cases per million inhabitants per year, characterized by the disappearance of hematopoietic tissue without abnormal cell proliferation. The ceased production of hematopoietic stem cells leads to a global failure of hematopoiesis resulting in peripheral cytopenias [1]. BMFS occur in young people, typically in the first three decades of life with a median age of 20 years, although a second peak occurs around the age of 60 years. These two ages of occurrence correlate with two different etiologies: a germline one as inherited BMFS (IBMFS) [2] is often diagnosed in younger patients whereas an acquired etiology, so-called acquired aplastic anemia (AA) or idiopathic aplastic anemia [3] predominates in older patients .

Acquired BMFS are the most frequent (80~85%) of BFMS, mainly accompanied by an autoimmune and more rarely (1~2%) a toxic or drug mechanism (such as benzene, pesticides, pharmaceutical drugs etc.). These so-called immunological forms include idiopathic aplastic anemia and post-hepatitis aplasia (hepatitis-aplasia syndrome). AA is also closely linked to paroxysmal nocturnal hemoglobinuria (PNH) [1,4,5].

Conversely, 15% to 20% BMFS are IBMFS which are more commonly seen in children (Table 2). However, the true incidence of each syndrome among IBMFS remain uncertain. There is a very wide variation in frequency between authors, possibly linked to the ethnicity, consanguinity rate and environmental influences. Historically, the most common etiologies are Fanconi Anemia and Diamond-Blackfan anemia [6,7]. On the other hand, the incidence of *GATA2* and *SAMD9/SAMD9L* syndrome in IBFMS remains difficult to determine because of their main identification at the myelodysplastic neoplasm (MDS)/acute myeloid leukemia (AML) stage [8]. Moreover, rare germline variants such as *MECOM and ERCC6L2*, that may occasionally present as BMF, have been recently identified [7,9] (see joint article on" germline predisposition ").

Indeed, inherited and acquired BMFS may progress towards MDS and AML [1,6]. Allogeneic hematopoietic stem cell transplantation (alloHSCT) offers much of the limited prospect of cure for all BMFS patients including those who do not respond to the immunosuppressive therapy (IST) used in acquired BMFS and can prevent evolution towards MDS or AML. Acquired cytogenetic or molecular abnormalities can be present before or at the onset of this progression and form part of the decision to perform alloHSCT.

In this review, we will discuss the diagnostic and prognostic relevance of cytogenetic abnormalities in acquired and inherited BMFS and propose recommendations for cytogenetic management at the time of diagnosis and follow up (Table 1).

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2. Clonal cytogenetic abnormalities in acquired BMFS

2.1. Acquired aplastic anemia

At diagnosis of AA, clonal chromosomal abnormalities (CAs), identified by karyotype (also called chromosomal banding analysis or CBA) performed on a bone marrow sample, are uncommon and are reported in about 4% to 15% of patients [10]. The most common CAs observed in AA are +8 (incidence 1.3%-6.7%) and -7 (incidence 2.0%-13.3%) (11–14). Monosomy 13/del(13q), +6 and +15 occur in less than 2% of cases; unlike in primary MDS, del(5q) is infrequent [5,15–19].

Monosomy 7 is associated with an increased risk of progression to MDS or AML, while +8 and del(13q) are linked with a more favorable response to IST and improved clinical outcome, mainly in terms of prolonged progression-free and overall survival as well as a lower risk of evolution into MDS or AML [5,17,20]. The high frequency of hypocellular bone marrow specimens can induce karyotype failure and underestimate the percentage of CAs in AA. The development of single nucleotide polymorphism array (aSNP) was helpful in terms of revealing subtle abnormalities below the level of CBA detection, with the supplementary advantage of its independence of cell culture. Notably, aSNP profiling identified a copy number loss of heterozygoty (cnLOH) in 6p as an uniparental disomy encompassing HLA genes and associated with a favorable response to IST [14,17,21,22]. Furthermore, 6p cnLOH may be of diagnostic importance, helping to distinguish between acquired and inherited BMFS, given it is present in 11%-19% AA patients but not found in IBMFS patients [23,24]. Overall, a 19% increase in the detection rate of CAs was obtained if CBA was combined with aSNP [14,22].

Secondary MDS as an evolution of AA is most commonly characterized by the presence of an isolated -7 or del(7q). The risk of secondary MDS/AML rises with disease duration, from 4%-8% to 9%-26% by 5 years and 10 years of follow-up, respectively [5]. As suggested in recent studies, the contribution of acquired mutations to the risk of malignancy in a given patient is still unclear, and has to be considered within the context of other patient- and disease-specific factors, but could be of value, especially in patients lacking prognostically relevant CAs such as -7 or del(7q) [5,17,25,26].

2.2. PNH disease

PNH is a clinically heterogeneous disease. In addition to the primary clinical manifestation of chronic intravascular hemolysis and thrombophilia, it can be associated with an AA. Interestingly, AA and PNH share a similar pathogeny linked to T-cell–mediated autoimmunity. These diseases can be associated at the time of diagnosis (AA/PNH) and each disease can appear during the evolution of the other. Nearly 40% of AA patients have a PNH clone at the time of diagnosis and 25% of PNH patients develop AA during follow-up [9,27].

PNH occurs as a consequence of a somatic mutation in the phosphatidylinositol glycan class A gene (*PIGA*) that impairs glycosylphosphatidylinositol (GPI) biosynthesis and results in deficient GPI-anchored complement regulatory proteins on the surface of mature blood cells, followed by clonal expansion of the mutated cells [28,29]. Flow cytometric methods allow the detection of GPI anchor-deficient clones at the time of diagnosis and monitoring of PNH clones. GPI-deficient granulocyte clone size varies widely among patients with PNH and may influence some clinical characteristics [28,30]. During the evolution of hemolytic PNH or AA/PNH towards secondary MDS or AML, the PNH clone dramatically decreases as the myeloid neoplasm clone expands [26]. Incidence of secondary MDS/AML at 15 years can reach 10% in patients with PNH [9].

PNH is generally considered as a disease with normal bone marrow karyotype. Most common CAs in PNH are similar to those observed in AA and include +8, del(13q), -7 and +6 [5,11,15] . Of note, del(13q), as in AA, is associated with a relatively benign clinical course [5,11,31]. Interestingly, an isolated del(13q) frequently occurs in patients with both PNH and AA, but is derived from PIGA wild-type cells, suggesting an alternative pathway of immune evasion [5,15]. Similarly to AA, del (13q) and +8 are linked to favorable responses to IST in PNH [15,32]. In contrast, -7 is of poor prognosis and considered to initiate a malignant transformation. Unlike in primary MDS, -7 is commonly isolated and may occur in the absence of somatic mutations [5,13,25]. Interestingly, rare cases of PNH with classical phenotype and auto-inflammatory features, but without BMF display a *PIGT*-PNH variant which induces a loss-of-function associated with a somatic del(20q) deleting the wild type PIGT [33]. Both PNH and AA patients need regular PNH clone screening and bone marrow investigations.

2.3. Differential diagnosis between AA and hypoplastic MDS

Differential diagnosis between AA (with or without PNH clone) and hypoplastic MDS (hMDS) may be difficult, particularly when CAs are absent. Conversely, identical CAs may be present in AA and hMDS. Diagnosis of AA requires documentation of depressed counts of at least two blood cell lineages and a hypocellular bone marrow. However, bone marrow hypocellularity may preclude an assessment of dysplastic changes and thereby a diagnosis of hMDS [34]. CAs are present in only ~50% of patients with MDS, and a normal karyotype does not exclude the diagnosis of MDS. Moreover, CBA may be uninformative in some hypocellular cases due to a lack of evaluable metaphases. In some cases, the presence of a CA is compatible with AA, although these abnormalities usually disappear after therapy. The presence of a PNH clone may also be helpful, as tiny PNH clones are encountered in 30% to 60% of idiopathic AA patients while PNH rarely coexist with primary MDS (~2%) [15,35].

Hypocellular MDS, classic MDS and AA show similarities as well as divergences in terms of somatic mutational pattern. Mutational patterns of hMDS seems to overlap with that of classic MDS, except for *SF3B1, SRSF2, ZRSR2,* and *U2AF1*, which are the typical drivers of mutations in classic MDS [12,25]. Recently, clonal myeloid mutations have also been found in a large proportion of patients with AA. [15,17,36]. Comparisons of the mutational spectra in AA versus hMDS show a similar pattern, but *BCOR/BCORL* mutations seem more frequent in AA whereas *TET2* mutations are less common [36,37]. Importantly, hMDS somatic patterns are distinct from those observed in secondary MDS evolved from AA: *RUNX1, SETBP1*, and *ASXL1* mutations and -7/del(7q) are more prevalent in post-AA MDS [25]. These mutations are related to clinical outcomes: mutations in *PIGA /BCOR/ BCORL1* are correlated with a better response to IST; mutations in a subgroup of genes that included *DNMT3A* and *ASXL1* were associated with worse outcomes [17,19,26,38]. Clinical morphological, cytogenetic, and mutational parameters were proposed to devise a diagnostic scoring system that allows for a formalized distinction between AA and hMDS [34].

3. Clonal cytogenetic abnormalities in inherited BMFS (IBMFS)

IBMFS is a group of heterogeneous disorders characterized by hematological cytopenias related to germline mutations and recognized as syndromes predisposed to myeloid neoplasms (see joint article).

GATA2 and *SAMD9/SAMD9L* syndromes are the most frequent germline syndromes with predisposition to myeloid neoplasms. They represent 7%-8% of children with primary MDS and are often associated with acquired -7. Germline *GATA2* variants are mutually exclusive with germline *SAMD9/SAMD9L* variants [8,39].

The other common IBMFS show disturbances in DNA repair, telomere biology, and ribosome biogenesis, which cause four major syndromes: Fanconi anemia, Dyskeratosis congenita, Diamond-Blackfan anemia, and Shwachman-Diamond syndrome [1,2,6]. Lastly, congenital amegakaryocytic thrombocytopenia is another type of IBMFS. IBMFS are typically diagnosed during childhood and occasionally in adults. Phenotype, genetics, hematological evolution and CAs found in IBMFS are summarized in Table 2.

3.1. GATA2 deficiency syndrome

GATA2 (GATA binding protein 2), which is located on the 3q21.3 band, encodes a zinc finger transcription factor with an essential role in transcription regulation of genes involved in hematopoiesis (https://www.ncbi.nlm.nih.gov/gene/), especially in hematopoietic stem cell activity, myeloid progenitor cell differentiation and erythroid precursor cell maintenance [40]. More than 180 germline nucleotide variants (inducing loss of function) or deletions have been identified and associated with *GATA2* deficiency syndrome [39]. It is a rare autosomal dominant genetic disease [41] with a high penetrance. These genetic abnormalities have been found in children or young adults with variable phenotype including hematological presentation and other clinical signs (lymphedema with Emberger syndrome, pulmonary alveolar proteinosis, hydrocele, congenital deafness and multiple abnormalities in other organ systems). Hematological phenotype is heterogeneous ranging from cytopenias and immunodeficiency to myeloid neoplasms [42] (Table 2). Monocytopenia and chronic neutropenia are frequent whereas anemia and thrombocytopenia are very rare. The prevalence of myeloid neoplasia in *GATA2* syndromes has been estimated to be 75%

Table 1
Characteristics and prognosis of recurrent cytogenetic abnormalities and FISH recommendations.

Diagnosis	Chromosomal abnormalities	Prognosis	Recommended FISH	References
Acquired BMFS at diagnosis				
BMFS +/- *myelodysplastic features (hSMD?)* +/- PNH clone*	+8 del(13q) -7 Other CAs	Good (IST) Good (IST) Poor Undetermined	None	(1,5,9,12–18,20,25,32,96,97)
	Normal karyotype	Undetermined	7q+cen7/cen8[§]+/-5q31(EGR1)[¤]	
	Karyotype failure [φ]		***Mandatory*** : 7q+cen7/cen8[§]+/-5q31(EGR1)[¤]	
PNH disease	*Normal karyotype (Majority of cases)*	Undetermined	None	(5,11)
	del(13q) +8 del(20q) -7 Other CAs	Good (IST) Good (IST) Undetermined Poor Undetermined		(5,9,11,13,15,25,31–33,97)
Inherited BMFS				
GATA2 syndrome deficiency	-7 del(7q) +8 and +21, other	Poor Poor Undetermined	***Mandatory*** : 7q+cen7[§]	(8,9,39,41,43,45)
	Normal karyotype	Undetermined		
	Karyotype failure [φ]			
SAMD9/SAMD9L syndrome	- 7/del(7q) CK	Variable according to clinical, hematological and cytogenomic course[Φ]	***Mandatory :*** 7q+cen7[§] with interphase quantification *(discordance between the percentage of -7 in nuclei and metaphases)*	(7–9,42,47,53,57)
	Normal karyotype			
	Karyotype failure [φ]			
Fanconi anemia [ϐ]	1q gain 3q gain -7/del(7q) del(20q) CK Other CAs	Undetermined Poor Poor Undetermined Poor Undetermined	***Mandatory*** : MECOM/7q[§]/RUNX1	(9,56,57,60,61,63–65,96,98)
	Normal karyotype	Undetermined		
	Karyotype failure [φ]			

(*continued on next page*)

Table 1 (*continued*)

Dyskeratosis congenita(DC)/Telomere biology disorders (TBDs)	Mainly normal karyotype -Y del(13q) - 7 CK	Undetermined Undetermined Poor Poor	None	(9,13,57,69)
	Karyotype failure ϕ		***Mandatory*** : 7q+cen7§	
Diamond-Blackfan anemia (DBA)	*Normal karyotype*	Undetermined	None	(9,57,72,73,75,76)
Shwachman-Diamond syndrome (SDS)	i(7)(q10) del(20q)	Undetermined	None	(9,57,84,86–89)
	Normal karyotype			
	Karyotype failure ϕ		***Mandatory*** : 7q+cen7§	
Congenital amegakaryocytic thrombocytopenia (CAMT)	*Rare*	Undetermined	None	(9,90,93,95)

Abbreviations: AA: aplastic anemia; CAs: chromosomal abnormalities; CK: complex karyotype; hMDS: hypoplastic MDS; IST: immunosuppressive therapy

*Including follow-up of AA with persistent cytopenia post IST or myelodysplastic features

ϕ Karyotype failure or insufficiently informative karyotype (less than 20 mitoses)

§ Probes for chromosome 7: cen7 and/or 7q probe(s). Probes for chromosome 8: cen8 and/or 8q probe(s) +/- control probe.

ϭ Presence of del(5q) suggests hMDS diagnosis

Φ High risk to acquire somatic leukemia driver gene mutations but spontaneous regressions are described

ϑ Diagnosis must have been confirmed on a chromosome breakage test.

with a mean age of 20 years at the time of diagnosis [8]. *GATA2* deficiency is observed in 10% of congenital neutropenia and/or BMF cases. An overlap with hMDS is possible. Monosomy 7 and del(7q) are the most frequent CAs and associated with poor prognosis. 50% of *GATA2* deficient cases with associated myeloid malignancies harbor del(7q) [41,43], mainly as an unbalanced translocation der(1;7)(q10;p10) [39,42,44,45]. *GATA2* deficiency has been identified as the most common hereditary cause of MDS in adolescents with -7 (72% of patients) [39].

Isolated +8 is the second CA occurring in approximately 20% of cases [39]. Other CAs such as +21 or +1q are widely reported [8,39]. Complex karyotype (CK) and del(5q) are generally not observed [8,39]. Somatic *GATA2* mutations are uncommon. Other somatic mutations can accumulate leading towards progression to MDS or AML (*ASXL1, RUNX1, TP53, GATA1, etc.*) [40]. It is worth noting that a somatic genetic rescue mechanism has been suggested in a familial case of *GATA2* deficiency [46].

3.2. SAMD9/SAMD9L syndromes

Germline heterozygote variants in *SAMD9/SAMD9L (SAMD9/9L)* syndrome are a recently described genetic predisposition to BMF and childhood MDS with -7, involving the particularity of a frequent somatic genetic rescue phenomenon [8,42,47,48]. *SAMD9* (sterile alpha motif domain containing) and its paralog *SAMD9L (SAMD9 like),* located in tandem at chromosome 7q21 are inflammatory inducible genes involved in the control of cell proliferation, antiviral response, tumor suppression and development [48]. *SAMD9/9L* syndromes occur frequently de novo, with autosomal dominant transmission, resulting from heterozygous gain of function variants, which are mostly missense variants [7,42,47]. Initially, a germline variant of *SAMD9* was reported in children with a severe multisystem disorder and monosomy 7 MDS called MIRAGE syndrome (Myelodysplasia, Infection, Restriction of growth, Adrenal hypoplasia, Genital phenotypes and Enteropathy) [49]. On the other hand germline *SAMD9L* variants were identified in patients with ataxia pancytopenia syndrome characterized by a progressive neurological phenotype, pancytopenia and hypoplasia [50] (Table 2). In *SAMD9/9L* syndrome, the incomplete penetrance is associated with various outcomes ranging from spontaneous hematological remission to MDS/AML. Several groups have reported the implication of *SAMD9/9L* variants in isolated MDS/AML and BMF with -7 [7,42,51,52]. Gain of function variants increase the effect of *SAMD9/9L* as a negative regulator of cellular proliferation. To escape the inhibitory effect of the variant, somatic genetic rescue can take place with progressive loss of the chromosome 7 or 7q that contains the mutated allele. Furthermore, several groups have documented a disappearance of −7 clones called transient monosomy 7 in young children [7,47,53].

Uniparental isodisomy 7q (UPD7q) and acquisition of somatic ''second hit'' loss of function mutation within the germline *SAMD9/9L* variant (g*SAMD9/9L*) are two other mechanisms of somatic genetic rescue, displaying benign courses. Furthermore, coexistence of independent populations with somatic *SAMD9/9L* mutation, UPD7q and isolated -7 and -7 with additional somatic mutations have been identified. Of note in the same study, healthy adults with g*SAMD9/9L* with no known rescue mechanisms have been identified through the involvement of other rescue mechanisms [42].

However, -7/del(7q) increases the risk of MDS/AML. Germline *SAMD9/9L* accounts for 8% of pediatric MDS patients. The g*SAMD9/9L* cases are mostly low blast MDS and present with -7 in 38%, CK in 5% and normal karyotype in 57% of cases. Somatic genetic rescue is detected in 61% of patients corresponding to 95% with -7/del(7q) and 51% with benign somatic genetic rescue [42].

Somatic mutations in leukemia driver genes are detected in 30% of cases and most frequently in patients with -7/del(7q): *SETPB1, ASXL1, RUNX1, EZH2, PTPN11, CBL and ETV6* according to Bluteau *et al.* [7] and are a major determinant of progression to MDS/AML [42].

In conclusion, prognosis of -7/del(7q) in *SAMD9/9L* syndrome is difficult to establish due to the varied and unpredictable evolution of the -7/del(7q) clones(9,42).

3.3. Fanconi anemia

Fanconi anemia (FA), one of the most common IBMFS, is a rare disease characterized by congenital abnormalities, chromosome fragility, progressive BMF, and susceptibility to cancer, such as MDS/AML and solid cancers (mostly head and neck squamous cell carcinomas) [54].

Transmission is autosomal recessive except for the very rare X-linked or autosomal dominant forms. The frequency of heterozygous subjects has been estimated at 1/300 in the United States and Europe. Twenty-two Fanconi genes have been identified (*FANCA-FANCW*).The most frequently mutated genes (90%) are *FANCA* (2/3 of cases), then *FANCG, FANCD2 and FANCC* (Table 2) [54]. FA is attributable to the biallelic inactivation of FA genes encoding associated proteins of the "FA core" multiprotein complex whose function is to control the repair of transcription errors and DNA cross-links damage in the S phase, leading to apoptosis via the *TP53* pathway [55].

10% to 20% of patients present a state of somatic reversion (correction of the mutation on one of the 2 alleles of the concerned FANC gene) that can delay diagnosis when it occurs in a hematopoietic stem cell, allowing improvement or even normalization of cytopenias [56]. The clinical phenotype is inconstant and highly variable: harmonious staturo-ponderal delay, facial dysmorphism, pigmented spots known as "café-au-lait", skeletal abnormalities mainly affecting the radius and thumbs (50% of cases), kidney and heart malformations (Table 2) [9,57].

In light of the numerous FANC genes and point mutations, as well as somatic reversion, cytogenetic and phenotypic tests remain the initial diagnostic tests. The chromosome breakage test is based on hypersensitivity to DNA interstrand crosslinking agents, such as mitomycin C, which increases spontaneous chromosomal breakage. This test which carried out on PHA stimulated blood lymphocytes may be normal in patients with somatic reversion [58]. The phenotypic test is the study of the mono-ubiquitination of FANCD2 by Western blot staining that can be performed on blood cells or fibroblasts. Molecular testing as a complement to these tests, permits either confirmation or exclusion of the diagnosis, even in somatic reversion cases [56,59,60].

Most FA cases present with hematological abnormalities (cytopenia). The median age of cytopenia onset is 7 years (0-36 years). At age 40, the cumulative incidence reaches 98%. Evolution is gradual towards a severe medullary insufficiency. Bone marrow smears show hypoplasia associated or not with aspecific dyserythropoiesis without prognostic value. Progression to a MDS or AML risk increases with age: 7% at 10 years, 27% at 20 years, and 43% at 30 years [61].

Frequency of acquired CAs increases with age: 15% at 10 years old, 37% at 20 years old, and 67% at 30 years old [61–63].

The most frequent and earliest CA is the 1q gain (+1q) (52%). This aberration is currently detected by karyotype, can present as 1q duplication, or unbalanced translocation, including jumping translocation, with various partner chromosomes, and breakpoint in the fragile pericentromeric 1q region [61,64]. The +1q can be observed at any abnormal hematological stage (BMF, MDS or AML). When isolated, +1q in non-MDS/AML categories is not a formal alloHSCT criteria, but requires regular cytogenetic monitoring [61,64].

Partial 3q gain (+3q) has a high incidence (40%) and present as partial trisomies or tetrasomies [61,64]. This anomaly is a strong indicator of poor prognosis in FA [65]. The candidate oncogene within the large 41.5-Mb common minimal duplicated region is *EVI1/MECOM*. This CA, found strictly in MDS/AML FA, can be cryptic, and thus detected by FISH or chromosome microarray [65].

Other CAs such as del(7q) or -7(30.6%), or the cryptic alterations of the *RUNX1* locus (22.6%) (such as the recurrent translocation t(1;21)(p36;q22)*/PRDM16::RUNX1*) are mainly found in MDS/AML FA and associated with poor prognosis. Less frequent CAs such as del(20q), del(11q), del(5q), and +8 are often found within a CK. This CK can harbor all the CAs mentioned above [61,64].

It must be noted that the presence of +1q and/or +3q in a child or young adult with MDS/AML, should raise concerns about an underlying diagnosis of FA. This is of particular importance as the therapeutic consequences would be major due to the high chemosensitivity of FA patients. [9,60,62].

3.4. Dyskeratosis congenita/telomere biology disorders

Telomere biology disorders (TBDs), including dyskeratosis congenita (DC), are caused by germline variants in telomere genes that result in very short telomeres. Telomeres are DNA-protein structures that protect chromosome ends from degradation and are essential for the maintenance of genome integrity [2]. When telomeres become critically short, chromosome ends are recognized as DNA double-strand breaks and DNA damage response pathways are activated, leading to apoptosis and senescence [66].

To date, at least 16 telomere genes have been identified (Table 2). 50% to 60% of patients have a variant in one of the six more frequently mutated genes: *DKC1 (15~20%), TINF2 (20%),TERC (5%), TERT, NOP10,* and *NHP2* [66] (Table 2).

Clinical presentation is dependent on the gene involved, the nature of the genetic anomaly, hetero- or homozygoty of the mutation, affected generation and environmental factors. Patients with DC/TBDs have variable genetic penetrance and expressivity and exhibit polymorphic clinical damages [66,67].

The classical phenotype of DC consists of the mucocutaneous triad of dysplasia (nail, oral leukoplakia, and reticular skin pigmentation). The patients are at very high risk of BMF, cancer and pulmonary fibrosis [2,66]. Childhood disease occurs with high penetrance, severe clinical symptoms, and specific organ involvement. BMF affects in young individuals (children, adolescents and young adults) and is often associated with hepatic or pulmonary damage. Isolated aplastic anemia, pulmonary fibrosis and liver disease occur predominantly in adults [66,68](Table 2).

Another recently identified adult form presents as an isolated MDS occurring earlier than in de novo MDS patients [9]. DC patients are subject to a 100-fold increased risk of AML that occurs in young patients. Telomere length is the major prognostic factor for evolution to MDS/AML and CAs acquisition [69,70].

Indeed, shorter telomeres at the time of diagnosis are associated with a more severe disease course, and an increased risk of clonal evolution to -7 (56%) [70] or CK and more rarely Y loss and del(13q) [69].

Constitutional telomerase gene mutations and rare polymorphisms are present in some AML patients without prior or concomitant BMF, and almost always display an aneuploid karyotype. Additionally, TBDs patients have recently been described as being of increased predisposition towards developing clonal hematopoiesis which is defined as a clonal expansion of somatic mutations in myeloid genes such as *DNMT3A, TET2*, and *ASXL1* within hematopoietic stem and progenitor cells, and a precursor stage for developing MDS/AML [71].

3.5. Diamond-Blackfan anemia

Diamond-Blackfan anemia (DBA) is a rare, congenital, and hypoplastic anemia that usually presents in early infancy. DBA is caused by a heterozygous allelic variation in 1 of the 20 ribosomal protein genes of either the small or large ribosomal subunit, ranging from point mutations to large deletions, which always results in haploinsufficiency of the relevant ribosomal protein (RP) gene such as *RPS19* (Table 2) and accounts for 20% of the mutations [72–76]. It is a model for ribosomal diseases and it was the first ribosomopathy ever described [75]. These deletions are usually seen in NGS (CNVs) but their detection may required other molecular technique such as MLPA and CGH. Of note, the cloning of a constitutional de novo balanced t(X;19)(p21;q13) translocation in a DBA child has led to the identification of this *RPS19* gene (19q13)[77].

Most DBA cases are sporadic, and inheritance is observed in 10% of patients with an autosomal dominant pattern. The phenotypic spectrum of DBA is very wide ranging from silent to severe. Physical anomalies are present in up to 50% of cases. These typically include growth retardation, craniofacial malformations, including hypertelorism, flat nasal bridge, and high arched or cleft palate. Thumb abnormalities are seen in 20% of patients, including the classical triphalangeal thumb which may also be suggestive of a FA diagnosis [75] (Table 2).

The hematological features are characterized by a moderate-to-severe macrocytic aregenerative anemia and occasional neutropenia or thrombocytosis. The bone marrow mostly shows an isolated erythroblastopenia. DBA patients are predisposed to myeloid malignancies and solid tumors, with a significantly lower risk than other IBMFS patients. Acquired CAs are rare findings in DBA and the bone marrow karyotype is mainly normal. Data in the literature about CAs preceding or accompanying AML and MDS are very scarce, preventing us from drawing generalities. Anyway, appearance of CAs during the follow-up of a DBA patient should raise concerns about a possible hematological malignancy [78,79]. In adulthood, screening for hematological changes to detect the occurrence of MDS and screening for solid tumors (especially osteosarcoma and colon cancer) are essential [79].

3.6. Shwachman-Diamond syndrome

Shwachman-Diamond syndrome (SDS) is a rare autosomal recessive disorder caused by mutations in the Shwachman Bodian Diamond

Table 2
Phenotype, genetics, hematological evolution and CAs in IBMFS.

Syndrome	Implicated genes : Transmission and estimated frequency	Clinical phenotype	Hematological and biological characteristics	MDS/AML characteristics	Acquired Cytogenetic Abnormalities	Somatic Mutations	Ref.
Fanconi anemia	AR: FANCA *(60%), FANCC (14%) , FANCD1 (BRCA2)(3%), FANCD2 (3%), FANCE (3%), FANCF (2%), FANCG (XRCC9)(10%), FANCI (1%), FANCJ (BRIP1)(2%), FANCL (PHF9), FANCM, FANCN (PALB2), FANCO (RAD51C), FANCP (SLX4), FANCQ (XPF), FANCR (RAD51), FANCS (BRCA1), FANCT (UBE2T), FANCU (XRCC2), FANCV (REV7/MAD2L2), FANCW (RFWD3)* AD: *FANCR (RAD51)* XLR : *FANC-B (2%)*	Variable phenotype: short stature, Intrauterine growth restriction (IUGR), triangular face, "Café au lait" spots, skeletal abnormalities (thumbs, fingers, forearm), kidney, heart, microcephaly, microphthalmia…	Isolated cytopenias or pancytopenia with hypoplastic or aplastic BM Increased chromosome breakage	MDS/AML occurring primarly at age 15 *(except FANCD1/BRCA 2* before age 5) Progression to AML/MDS increasing with age (15% at 10, 67% at 30)	+1q and +3q gain involved in unbalanced translocations, cryptic 21q translocations (*RUNX1*) Hish risk : del(7)q, -7, +3q, *RUNX1* tranlocations, and CK	*RUNX1 and RAS* pathway mutations (high risk) Somatic reversion of FANC genes	(9,56,57,60,61,63–65,96,98)
Dyskeratosis congenita/telomere biology disorders	XLR: DKC1 (15-20%) AD: TINF2*(TIN2)*(11-20%),*TERC* (5%), *NAF1* AR: *CTC1,USB1, NOP10, TCAB1, NHP2,POT1* AD & AR:*TERT, RTEL1*(5-10%), *ACD, PARN* Unknown in 30% of cases	Classical muco-cutaneous triad: nail dystrophy, oral leukoplakia, skin pigmentation Pulmonary and liver fibrosis	Isolated cytopenias or pancytopenia with hypoplastic or aplastic BM	hMDS, MDS (3%, probably undervalued) AML secondary to MDS	Various High risk: -7, del(7q), CK	Various High risk : *TP53** mutation	(9,13,57,69)
Diamond-Blackfan anemia	AD: *RPS7,* RPS10 (2%*), RPS15, RPS17 (1%), RPS19 (25%), RPS24 (2%), RPS26 (10%), RPS27, RPS28, RPS29 A, RPL5 (7%), RPL9, RPL11 (6%), RPL15, RPL18, RPL26 (6%), RPL27, RPL31, RPL35A(3%)* AR: HEATR3 XLR: *TSR2*	Short stature, IUGR, craniofacial, cardiac, genitourinary, thumb and bone malformations (50% of patients)	Early and symptomatic macrocytic aregenerative anemia with erythroblastopenia (first year of life) Possible moderate neutropenia or thrombocytopenia Rare typical aplasia	MDS and secondary AML after 30 years	Rare	*TP53** and *ASXL1* mutations (rare)	(9,57,72–76)

(*continued on next page*)

Table 2 (*continued*)

Shwachman-Diamond syndrome	AR: *SDBS* (90%), *DNAJC21, EFL1* AD: *EIF6, SRP54 (<10%)*	Exocrine pancreatic insufficiency Hepatomegaly Skeletal abnormalities (bone dysplasia and osteoporosis…)	Frequent isolated neutropenia and/or anemia Pancytopenia with hypoplastic or aplastic BM Common aspecific dysmyelopoiesis without MDS	MDS/AML : 20% at 30 years, AML with high risk	Clonal abnormalities without prognostic value (somatic genetic rescue) : isochromosome 7q and del(20q)	Biallelic *TP53** mutation EIF6 mutation (improves ribosome biogenesis efficiency)	(9,57,84,86–89)
Congenital amegakaryocytic thrombocytopenia	AR : *MPL*	Rare reported malformations with undetermined causality	Neonatal severe thrombocytopenia usually, absence of megakaryocytes, frequent progression to pancytopenia Elevated TPO rate	undescribed	undescribed	undescribed	(9,90,93,95)
	AR : *THPO*	undescribed	Moderate to severe thrombocytopenia (child/young adult), anemia and neutropenia of variable severity Very low TPO rate	undescribed	1 case of monosomy 7 during evolution	undescribed	
SAMD9/ SAMD9L syndrome	AD: *SAMD9, SAMD9L* (both located on 7q)	*SAMD9*: MIRAGE syndrome (MDS-infections-growth retardation-adrenal hypoplasia-genital abnormalities-enteropathy) *SAMD9L*: ATXPC (pancytopenia ataxia syndrome) variable neurological abnormalities with constant cerebellar atrophy, peripheral neuropathy, ophtalmologic abnormalities…	Cytopenia with hypoplastic BM occurring during the first 2 decades Variable profile but characterized by somatic mosaicism with chromosome 7q UPD or inactivating mutations of the mutated SAMD9 or SAMD9L alleles, causing spontaneous cytopenia correction	MDS and/or AML	Monosomy 7 or del(7q) that can be transient (corrected by somatic genetic rescue) or can persist and lead to MDS The prognostic value of monosomy 7 or del(7q) should be discussed	*SETBP1, RUNX1, ETV6, ASXL1* somatic mutation in leukemic clones	(7,9,42,47,53,57)
GATA2 deficiency	AD: *GATA2*	Immune deficiency : predisposition to HPV/bacterial/atypical mycobacterial infections,lymphedema, vascular malformations, deafness…	Pancytopenia (neutropenia and monocytopenia in particular), hypoplastic BM, rare typical aplasia, frequent moderate dysplasia	MDS/AML : 70% at 70 years	Monosomy 7, del(7q), +8, +21q… High risk : monosomy 7	*ASXL1* and *SETBP1* somatic mutations reported as pejorative	(8,9,39,41,43,45)

Frequency not given for genes that are rarely involved

Abbreviations : AD : autosomal dominant ; AML : acute myeloid leukemia ; AR : autosomal recessive; BM : bone marrow ; MDS : myelodysplastic neoplasm ; hMDS : hypoplastic MDS ; UPD : uniparental disomy ; XLR : X-linked recessive

* TP53 mutation may also described in DBA-like and Dyskeratosis congenita not only a somatic mutation (74)

Syndrome (*SBDS*) gene in at least 90% of cases; while 4 other genes have been associated with a SDS-like phenotype: *DNAJC21, EFL1, SRP54 and EIF6.* All these genes are involved in ribosome maturation. Biallelic mutations of the *SBDS* or the *EFL1* genes impair release of the anti-association factor EIF6 form the 60S ribosomal unit, a key step in the translational activation of ribosomes. SDS is therefore a ribosomopathy characterized by exocrine pancreatic insufficiency, poor growth, skeletal abnormalities, and BMF, with neutropenia as the most prominent feature [80] and an increased risk of MDS and AML [2]. 15% to 20% of patients with SDS develop a MDS with a high risk of AML transformation and poor prognosis [81–84]. The most frequently observed CAs are an isochromosome of the long arm of chromosome 7, i(7)(q10), and an interstitial deletion of the long arm of chromosome 20, del(20q) [85]. Both abnormalities are prognostically benign as they are not associated with leukemia progression. Indeed, it has been recently demonstrated that these abnormalities represent a somatic genetic rescue. Isochromosome 7q duplicates the hypomorphic *SBDS* allele (7q11.2) thereby allowing a higher level of SBDS protein production whereas del(20q) causes the loss of one *EIF6* allele (20q11.2) leading to a reduced level of the *EIF6* protein thus improving protein ribosome biogenesis. In the same way, somatic genetic rescue can occur through *EIF6* acquired somatic mutations [57,86–88]. Conversely, transformation into MDS/AML is frequently associated with a CK and /or a biallellic *TP53* mutation [84–86,89]. Therefore bone marrow karyotype and targeted NGS remain important tools during disease follow-up [84].

3.7. Congenital amegakaryocytic thrombocytopenia

Congenital amegakaryocytic thrombocytopenia (CAMT) is a rare IBMFS characterized by severe thrombocytopenia at birth due to

ineffective megakaryopoiesis and development towards BMF during the first few years of life. [90]. CAMT is mainly caused by mutations in the gene encoding the thrombopoietin receptor (*MPL*) and less frequently in the gene encoding its ligand (*THPO*) [90] (Table 2). On the other hand, CAMT in association with radio-ulnar synostosis, which is not always clinically apparent, is mostly caused by mutations in *MECOM* [91], and rarely in *HOXA11* [6]. Development of BMF due to exhaustion of early hematopoietic progenitors is a characteristic feature of CAMT-MPL and reveals the essential role of *MPL* in early hematopoiesis [92]. All CAMT-MPL patients show a progression towards a hypoplastic bone marrow with pancytopenia [90]. CAs in CAMT-MPL bone marrow are very rare [90,93,94] including one case with -7 [95] and only a single patient was reported to have developed overt leukemia [90].

4. CBA interpretation and FISH strategies in acquired and inherited BMFS (Table 1)

We consider that chromosomal banding analysis (CBA/karyotype) is mandatory for all BMFS at the time of diagnosis [9,57,96]. Firstly, it has a theranostic value in acquired BMFS with autoimmunity such as AA/PNH given +8 and del(13q) show a good response to IST. Consequently, the +8 FISH is recommended in case of karyotype failure [97]. Follow-up of AA/PNH and post-IST AA with cytogenetic bone marrow analysis is recommended every 12 to 18 months [9]. Secondly, due to the strong diagnostic impact of -7 in all BMFS (acquired and inherited), the use of 7q probes is recommended in case of normal or karyotype failure in BMFS with dysmyelopoiesis, with or without PNH clone, and also in DC/TBDs, and in SDS [57]. Finally, for differential diagnosis between AA with myelodysplastic features and hMDS, the FISH 5q can be also used in case of karyotype failure [97].

The characteristic +3q, del(7q) and *RUNX1* rearrangement seen in FA evolution and its prognostic impact implies mandatory karyotype and FISH at the time of diagnosis and every year, using probes for chromosome 7q, 3q (*MECOM*) and a break-apart *RUNX1* whatever the CBA result [9,57,61,63,64,98].

Cytogenetic evaluation in *GATA2* and *SAMD9/9L* syndromes is recommended every 12 to 18 months [9]. CBA with interphase FISH is mandatory to evaluate -7/del(7q) clones.

Cytogenetic monitoring of SDS is carried out on a regular basis during disease follow-up with no precise timing [84].

CBA of DC/TBDs are monitored every 18 months or earlier in the event of worsening myelogram results [9].

Cytogenetic follow-up is not recommended in CAMT and DBA due to the virtual absence of CAs in these disorders (Table 1).

5. Technical recommendations from GFCH

The recommended bone marrow culture time concerning CBA analysis is at least overnight. The particularity of the BMFS cell culture is karyotype failure as a result of bone marrow sample hypocellularity linked to the BMFS. Adding myeloid lineage stimulants such as G-CSF can partly overcome these failures with a 3-day culture peak of activity. FISH is mandatory in case of karyotype failure. FISH can complement CBA as proposed in Table 1, according to CA frequency and prognosis value, the existence of cryptic CAs and bone marrow morphological features.

It should be noted that the contribution of new technologies such as optical genome mapping which requires a low quantity of cells could be interesting in terms of BMFS but needs to be evaluated (see joint article “guidelines new technologies”).

6. Conclusion

Clonal hematopoiesis in acquired and inherited BMFS is commonly observed in younger patients. These patients need prolonged bone marrow monitoring including morphological, cytogenetic and molecular evaluation. Morphological progression or the occurrence of acquired CAs or molecular abnormalities can prompt clinicians to consider alloHSCT in eligible patients.

The chromosomal abnormalities found in BMFS are not specific and can not be used to distinguish IBFMs, acquired BMFS or hypoplastic MDS. Only a cluster of clinical and biological arguments (flow cytometry, cytogenetics, molecular biology, etc) can allow diagnosis.

Monosomy 7 and del(7q) are strong indicators of germline predisposition in pediatric MDS and BMFS [42] and remain of poor prognosis in all BMFS with the exception of *SAMD9/9L* syndrome where they can be difficult to interprete as they can be representative of a somatic genetic rescue mechanism. CAs concerning acquired BMFS (+/-PNH), such as +8 and del(13q) remain good prognosis markers for IST response. For hematopoietic evolution of FA, there are characteristic CAs with variable but important prognostic significance.

The emergence of newer genomic sequencing and other techniques is incrementally helpful to elucidate the mechanisms of this genetically heterogeneous group of diseases in terms of detection, and offers the potential to improve patients outcome. However, the landscape of somatic mutations is complex and incompletely understood. Morphological, cytogenetic, and molecular data should be carefully interpreted to make the appropriate therapeutic decisions. The crucial role of cytogenetic monitoring is to evaluate an earlier transformation in MDS or AML ascertaining the best time for non- transplant treatment or alloHSCT.

CRediT authorship contribution statement

Wendy Cuccuini: Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Conceptualization. **Marie-Agnes Collonge-Rame:** . **Nathalie Auger:** Writing – original draft, Resources. **Nathalie Douet-Guilbert:** Writing – original draft, Investigation. **Lucie Coster:** Writing – review & editing. **Marina Lafage-Pochitaloff:** Writing – review & editing, Writing – original draft, Validation, Formal analysis, Conceptualization.

Declaration of Competing Interest

None.

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Wendy Cuccuini[a,*], Marie-Agnes Collonge-Rame[b], Nathalie Auger[c], Nathalie Douet-Guilbert[d], Lucie Coster[e], Marina Lafage-Pochitaloff[f]

[a] *Laboratoire d'Hématologie, Unité de Cytogénétique, Hôpital Saint-Louis, Assistance Publique Hôpitaux de Paris (APHP), 75475, Paris Cedex 10, France*

[b] *Oncobiologie Génétique Bioinformatique UF Cytogénétique et Génétique Moléculaire, CHU de Besançon, Hôpital Minjoz, 25030, Besançon, France*

[c] *Laboratoire de Cytogénétique/Génétique des Tumeurs, Gustave Roussy, 94805, Villejuif, France*

[d] *Laboratoire de Génétique Chromosomique, CHU Brest, Hôpital Morvan, 29609, Brest Cedex, France*

[e] *Laboratoire d'Hématologie, Secteur de Cytogénétique, Institut Universitaire de Cancérologie de Toulouse, CHU de Toulouse, 31059, Toulouse Cedex 9, France*

[f] *Laboratoire de Cytogénétique Hématologique, CHU Timone, Assistance Publique Hôpitaux de Marseille (APHM), Aix Marseille Université, 13005, Marseille, France*

[*] Corresponding author at: Laboratoire d'Hématologie/Unité de Cytogénétique, Pôle Biologie Pathologie Physiologie, Hôpital Saint-Louis, 1 avenue Claude Vellefaux, 75475, Paris, Cedex 10, France.
E-mail address: wendy.cuccuini@sls.aphp.fr (W. Cuccuini).