Nordic Guidelines for Germline Predisposition to Myeloid Neoplasms in Adults

Recommendations for Genetic Diagnosis, Clinical Management and Follow-up

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Nordic Guidelines for Germline Predisposition to Myeloid Neoplasms in Adults: Recommendations for Genetic Diagnosis, Clinical Management and Follow-up

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Abstract

Myeloid neoplasms (MNs) with germline predisposition have recently been recognized as novel entities in the latest World Health Organization (WHO) classification for MNs. Individuals with MNs due to germline predisposition exhibit increased risk for the development of MNs, mainly acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS). Setting the diagnosis of MN with germline predisposition is of crucial clinical significance since it may tailor therapy, dictate the selection of donor for allogeneic hematopoietic stem cell transplantation (allo-HSCT), determine the conditioning regimen, enable relevant prophylactic measures and early intervention or contribute to avoid unnecessary or even harmful medication. Finally, it allows for genetic counseling and follow-up of at-risk family members. Identification of these patients in the clinical setting is challenging, as there is no consensus due to lack of evidence regarding the criteria defining the patients who should be tested for these conditions. In addition, even in cases with a strong suspicion of a MN with germline predisposition, no standard diagnostic algorithm is available. We present the first version of the Nordic recommendations for diagnostics, surveillance and management including considerations for allo-HSCT for patients and carriers of a germline mutation predisposing to the development of MNs.

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Introduction

Although the majority of myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML) cases are sporadic, the introduction of next-generation sequencing (NGS) into the diagnostic work-up has revealed that hereditary MDS or AML are more common than previously thought. Estimates suggest that about 5% to 15% of adults and 4% to 13% of pediatric patients with MDS or AML carry germline pathogenic variants in cancer susceptibility genes.[1–4] At the same time, several new genes associated with familial MDS or AML, with or without syndromic features, have been recently discovered, such as *GATA2*, *ETV6*, *DDX41*, *SAMD9,* and *SAMD9L*.[5–20]

In 2016, *myeloid neoplasms with germline predisposition* were included as a new dedicated entity in the revision of the WHO classification of myeloid neoplasms (Table 1), thus acknowledging the clinical importance of recognizing these disorders during the diagnostic work-up of patients with MN.[21] Consideration of MDS or AML germline predisposition syndromes has also been integrated in the clinical management guidelines of patients with MDS or AML of the European Leukemia Net and the National Comprehensive Cancer Network.[22,23] In addition, experts in this field have provided recommendations on which patients should be investigated for germline predisposition syndromes, and how such patients and families should be managed.[24–31] For specific disorders such as Fanconi anaemia,[32] Shwachman-Diamond syndrome,[33] Diamond-Blackfan anaemia,[34] and telomere biology disorders[35] consensus guidelines already exist, but for the other rare disorders predisposing to MDS and AML, international recommendations and guidelines for adult patients have not yet been developed.[36]

Recognition of a specific germline predisposition in a patient with MDS or AML is important not only for psychological reasons providing an explanation for the disease, but also because of clinical implications as it may tailor therapy, dictate the selection of donor for allogeneic hematopoietic stem cell transplantation (allo-HSCT) and determine the conditioning regimen.[37] For disorders with extra-hematopoietic manifestations, a molecular diagnosis may enable prophylactic measures, early intervention or contribute to avoid unnecessary or even harmful medication.[38] Finally, it allows for genetic counseling and follow-up of at-risk family members.[38]

The rarity of hereditary MDS and AML combined with the heterogeneous clinical presentation makes identification of these patients challenging. For this reason, the Nordic MDS study group (NMDSG) decided to establish a working group with the purpose of creating and implementing common Nordic clinical guidelines to ensure uniformity in diagnostic procedures and management of patients and their family members at risk. The working group members are hematologists, pediatricians, and clinical/medical geneticists from Sweden, Norway, Finland, and Denmark. The initial focus of the working group was on the adult patients with MDS or AML and a germline predisposition, but hopefully these guidelines may also be of value in other settings.

This document is based on a review of the current literature and presents the first version of Nordic recommendations for diagnostics, surveillance and management including considerations for allo-HSCT for adult patients and carriers of a germline mutation predisposing to MDS or AML. It will be also posted on the NMDS website (NMDS.org) and updated regularly.

Which patients should be tested for germline conditions predisposing to myeloid neoplasms

Who should test patients suspected for germline disorders

Diagnostic genetic testing for germline variants in patients with MDS or AML may be performed by the clinical/medical geneticist as part of genetic counseling or requested by a specialized hematologist without prior referral to genetic counseling, depending on the legislation of each country. As the genetic investigation of suspected myeloid germline disorders is challenging, close collaboration between the hematologist who is more likely to identify these patients and the geneticist responsible for the actual testing, is mandatory. However, it is important that genetic counseling is offered to all patients investigated for potential germline conditions, even if no pathogenic variant is detected.

Genetic counseling is highly recommended prior to genetic testing of all apparently healthy relatives for germline predisposition for myeloid neoplasms (predictive and presymptomatic testing) including predictive testing of HLA-identical potential family donors.

Criteria for whom to test

For the development of the following criteria a number of factors have been considered, namely:

- Age
- Family history
- Personal medical history
- Clinical/physical findings
- Genetic characterization of the clonal cells
- Recommendations published in the recent literature
- Our clinical experience thus far.

A: Patients with positive family history or signs/symptoms indicative of a hereditary condition predisposing to myeloid neoplasms (MN) especially MDS and AML.

A1: Patient with MDS or AML and symptoms/signs of a hereditary condition predisposing to MN development[1] diagnosed before the age of 50.
A2: Two individuals (first or second-degree relatives, FDR, and SDR, respectively) with MDS or AML or long-lasting thrombocytopenia or symptoms/signs indicative of a hereditary condition predisposing to MN development[1], one of whom diagnosed before the age of 50.
A3: One individual with MDS or AML and two FDR or SDR with a diagnosis of solid tumor malignancy[2] one of whom diagnosed before the age of 50.

[1] Excessive toxicities with chemotherapy or radiation, multiple cancer diagnoses, therapy-related leukaemia, poor stem cell mobilization of a sibling candidate donor (reported in rare cases related to *TERC* and *RUNX1* germline variants)[27,39], consanguinity, skin or nail abnormalities, unexplained liver disease, pulmonary fibrosis or alveolar proteinosis, short stature, microcephaly or characteristic skeletal abnormalities or other congenital abnormalities, Café au lait spots, hypopigmented macules, lymphedema, immune deficiencies, atypical infections, excessive warts (Table 1).

[2] other haematological malignancies or cancer forms suggestive of constitutional mismatch repair deficiency syndrome, Li-Fraumeni syndrome, *BRCA2* related syndromes (such as sarcomas, adrenocortical carcinomas, brain tumours, gastrointestinal, genitourinary, breast, ovarian and pancreas cancer). It should be noted that a number of conditions included in criterion A may appear with atypical or no clinical stigmata, therefore a detailed personal and family history including a three generations pedigree is needed. In order to facilitate the obtainment of such a history a questionnaire proposed by Churpek et al.[30] from the Chicago group could be used.

Table 1

Overview of germline conditions predisposing for myeloid neoplasms (adapted from WHO 2016 book chapter and NCCN MDS v1.2019)

Genetic syndrome	Gene(s)	Inheritance pattern(s)	Characteristic haematological malignancies	Lifetime risk for myeloid malignancies	Other phenotypes and clinical features	Diagnostic testing
Myeloid neoplasms with germline predisposition without a pre-existing disorder or organ dysfunction						
Acute myeloid leukemia with germline *CEBPA* mutation	*CEBPA*	AD	AML	>80%	–	DNA sequencing including del/dup analysis
Myeloid neoplasms with germline *DDX41* mutation	*DDX41*	AD	MDS, AML	Unknown, probably high but mostly in older age	CML, CMML and lymphomas have also been reported	DNA sequencing
Chromosome 14q32 duplication syndrome	14q32 genomic duplication	AD	AML, MPNs, CMML	High penetrance in the 5 families reported	–	Del/dup analysis
Myeloid neoplasms with germline predisposition and pre-existing platelet disorders						
Myeloid neoplasms with germline *RUNX1* mutation (Familial platelet disorder with associated myeloid malignancy)	*RUNX1*	AD	MDS, AML	~45%	Thrombocytopenia and abnormal platelet function; clonal hematopoiesis; ALL	DNA sequencing including del/dup analysis
Myeloid neoplasms with germline *ANKRD26* mutation	*ANKRD26*	AD	AML, MDS, CML	8%	Moderate thrombocytopenia with mild bleeding manifestations	DNA sequencing of 5'UTR
Myeloid neoplasms with germline *ETV6* mutation	*EVT6*	AD	ALL, AML, MDS	Unknown	Thrombocytopenia and mild bleeding manifestation	DNA sequencing
Myeloid neoplasms with germline predisposition and other organ dysfunction						
GATA2 deficiency syndrome	*GATA2*	AD	MDS, AML	>80%	Immunodeficiency (B-/NK-/CD4-cell lymphocytopenia, monocytopenia), susceptibility to viral infections, warts, disseminated nontuberculous mycobacterial infections, lymphedema, sensorineural hearing loss, pulmonary alveolar proteinosis	DNA sequencing (including intronic regions) and del/dup analysis
MIRAGE syndrome	*SAMD9*	AD	MDS, AML with monosomy 7	High, spontaneous resolution through revertant mosaicism possible	Cytopenias and marrow failure; growth restriction, infection susceptibility, adrenal hypoplasia, genital phenotypes, and enteropathy.	DNA sequencing
Ataxia-pancytopenia syndrome	*SAMD9L*	AD	MDS, AML with monosomy 7	High, spontaneous resolution through revertant mosaicism possible	Cytopenias and marrow failure; gait disturbance, nystagmus, cerebellar atrophy and white matter hyperintensities; immunodeficiency	DNA sequencing
Bone marrow failure syndrome 1 (BFMS1/SRP72)	*SRP72*	AD	MDS	Unknown	Congenital sensorineural deafness	DNA sequenicng
Fanconi anaemia	*FANCA*	XLR	MDS, AML	~10%	Bone marrow failure, short stature, skin pigmentation (café-au-lait or hypopigmented spots), skeletal anomalies (thumbs, arms), congenital heart disease, ear anomalies, renal malformations, squamous cell carcinomas)	DNA sequencing including del/dup analysis Chromosomal breakage analysis
	FANCB, FANCC, BRCA2, FANCD2, FANCE, FANCF, FANCG, FANCI, FANCM, PALB2, RAD51C, SLX4	AR				
Severe congenital neutropenia	*ELANE, CSF3R, GFI1, SRP54*	AD	MDS, AML	21–40%	Severe neutropenia	DNA sequencing including del/dup analysis
	HAX1, G6PC3, JAGN1, VPS45	AR				
	WAS	XLR				
Shwachman-Diamond syndrome	*SBDS*	AR	MDS, AML, ALL	5–24%	Neutropenia, pancreatic insufficiency, short stature, skeletal abnormalities	DNA sequencing including del/dup analysis
Diamond-Blackfan anaemia	*RPS19, RPS17, RPS24, RPL35A, RPL5, RPL11, RPL15, RPL26, RPS7, RPS26, RPS10, RPS29*	AD	MDS, AML, ALL	~5%	Anemia and marrow erythroid hypoplasia. Small stature, congenital anomalies (e.g. craniofacial, cardiac, skeletal, genitourinary)	DNA sequencing including del/dup analysis Elevated erythrocyte adenosine deaminase
	GATA1	XLR				

(*continued*)

Table 1
(continued).

Genetic syndrome	Gene(s)	Inheritance pattern(s)	Characteristic haematological malignancies	Lifetime risk for myeloid malignancies	Other phenotypes and clinical features	Diagnostic testing
Telomere biology disorders	*DKC1*	XLR	MDS, AML	2–30%	Bone marrow failure, nail dystrophy, abnormal skin and pigmentation, oral leukoplakia, early hair graying, pulmonary fibrosis, hepatic fibrosis, squamous cell carcinoma	DNA sequencing including del/dup analysis Telomere length analysis
	TERT, TERC, TINF2, RTEL1, PARN, ACD	AD				
	NOP10, NHP2, WRAP53, RTEL1, TERT, CTC1,PARN, ACD	AR				
Down syndrome	Trisomy 21	95% De novo, 5% translocation	Transient abnormal myelopoiesis/ AML, Acute megakaryoblastic leukemia, ALL	10% (transient abnormal myelopoiesis) ~2–3% ALL, AML	Down syndrome: multiple congenital anomalies, dysmorphic features, intellectual disability	Karyotype
RASopathies	*CBL, KRAS, NF1, PTPN11*	AD	JMML, AML	~10%	Short stature, facial features, cardio-thoracic defects, coagulopathy	DNA sequencing
Constitutional mismatch repair deficiency	*MLH1, MSH2, MSH6, PMS2, EPCAM*	AR	ALL, lymphomas, AML, MDS	Unknown, risk ~30% for lymphoma/ALL	Café-au-lait spots, brain tumors, colorectal cancer, osteosarcoma, and other solid tumors.	DNA sequencing including del/dup analysis
Bloom syndrome	*BLM*	AR	ALL, AML/MDS, lymphoma	15%	growth deficiency, photosensitive skin changes, immunodeficiency, early-onset diabetes, microcephaly, high-pitched voice, hypogonadism, risk for other cancers	DNA sequencing including del/dup analysis
LIG4 syndrome	LIG4	AR	MDS	Rare (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6096036/)	Short stature, microcephaly, Immunodeficiency combined; pancytopenia & myelodysplastic syndrome	DNA sequencing including del/dup analysis
Li Fraumeni syndrome	*TP53*	AD	ALL, MDS, AML	2–4%	High risk for cancer (50% by age 30 years and 90% by age 60 years) especially high risk for adrenocortical carcinoma, brain cancer, breast cancer, choroid plexus carcinoma, colon cancer, lung carcinoma, sarcoma, other tumors.	DNA sequencing including del/dup analysis
Other bone marrow failure syndromes	*MECOM*	AD	MDS, AML	Unknown	Skeletal/cardiac abnormalities, neurological defects	DNA sequencing
	ERCC6L2	AR				

AD = autosomal dominant; ALL = acute lymphoblastic leukemia; AML = acute myeloid leukemia; AR = autosomal recessive; CML = Chronic Myeloid leukemia; CMML = chronic myelomonocytic leukemia; JMML = juvenile myelomonocytic leukemia; MDS = myelodysplastic syndrome; XLR = X-linked recessive. Risks for development of myeloid malignancies have been based on: The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia, McGee et al 2016, ASH educational book 2016/2017 and GeneReviews (https://www.ncbi.nlm.nih.gov).

A4: ≥3 FDR or SDR with MN or long-lasting thrombocytopenia or symptoms/signs indicative of a hereditary condition predisposing to MN development[1], independently of age.

B: Patients with MN where the diagnostic work-up for the determination of the somatic genomic background has detected gene variants suspected to be germline (near heterozygous or near homozygous).

A number of genes that causes hereditary MN can also be somatically mutated in sporadic cases. Classical examples are variants within the *RUNX1, GATA2*, *TP53, ETV6* and *CEBPA* genes.[40,41] Analysis of such variants with the advent of NGS panels is performed routinely as part of the diagnostic work-up for MDS and AML patients, especially in young individuals. The Variant Allele frequency (VAF) of a specific variant detected on the somatic NGS panel can suggest that the variant may be of germline origin, when the VAF is 40% – 60% [near-heterozygous] or >90% [near-homozygous].[4,42] In such cases further testing of extra-hematological tissue for the respective variant is highly recommended after obtaining the patient's consent. The detection of a pathogenic germline variant through a somatic gene panel is more likely in younger patients with MDS or AML (<50 years) but may also be identified in older age groups. Particularly, variants in the *DDX41* gene are associated with MDS and AML between 55 and 65 years of age. Furthermore, the type of variant may be of importance, for example truncating variants in the *DDX41* gene are usually of germline origin.[12,13]

It should be highlighted that the majority of the NGS panels used in the current clinical setting are designed for the detection of somatic variants, therefore a normal result does not preclude the possibility of a germline variant in regions not included in the actual analysis.

C: Patients not fulfilling the criteria A and B diagnosed with MDS or AML before the age of 50 carrying aberrations of chromosome 7 [monosomy 7/del(7q)/der(7)].

A family or personal history without any suspicion of a hereditary disorder does not exclude an underlying predisposing germline variant.[43] *De novo* mutations, gonadal mosaicism, genetic reversion, variable penetrance and expressivity may explain the absence of distinctive clinical features. It is well established that early cancer debut strongly implies heredity.[1] Several reports in the literature favor genetic testing for hereditary conditions predisposing to MN for all young patients.[5,43] That said and for the time being, our working group proposes that among young patients (<50 at diagnosis) without a family or personal history only those with aberrations of chromosome 7 (monosomy 7/del(7q or other aberrations with loss of 7q material), which is particularly common in *GATA2*- and *SAMD9/SAMD9L*-related disorders[16,17,44–46] should be further referred for genetic counseling/testing.

Preliminary data suggest that specific somatic gene variants may likewise be over-represented in some disorders predisposing to MN.[10,47–49] They may in the future prove to be useful indicators to identify patients with MN who do not meet the standard criteria for genetic testing for a predisposing germline variant.[49]

We think that testing all young patients independently of family/personal history could be performed primarily in the context of clinical trials rather than in the clinical setting, at least for the time being. If, however, resources are available and national guidelines approve, genetic counseling/investigation for MDS or AML predisposing syndromes could be offered to all young patients.

Limitations of the proposed criteria to take into consideration

It is important to highlight that the current criteria are focused mainly on known genetic predisposition syndromes to MDS or AML. The proposed age threshold of 50 years at the time of diagnosis is arbitrary and conditions such as predisposition to MDS or AML due to germline *DDX41* mutations[13] or patients debuting at a later age than expected may be underrepresented. Moreover, a number of genetic aberrations predisposing to the development of myeloid neoplasms may have not yet been identified; therefore, some patients may not fit the aforementioned criteria. However, they may still be eligible for genetic counseling/investigation. In case of any clinical suspicion of a hereditary condition not included in the following criteria, a referral to an institution with expertise in the field is recommended.

How should patients with MN and suspicion of germline predisposition be genetically investigated

Different approaches to genetic testing exist between the Nordic countries and even within each country. This is primarily due to various organizational structures and access to funding. Genetic testing should be performed with the aim to detect both single nucleotide variants (SNVs) and copy-number variations (CNVs). Taking into consideration the number of the investigated genes, comprehensive genetic analysis for these rare diseases can be only performed with the advent of NGS, with the prerequisite that such a technology is available in the clinical setting. It is not our goal to provide guidelines on the actual method that should be used, as long as the genetic testing is performed following validated and accredited methodologies. Instead, we propose a number of genetic conditions that should be assessed in all patients fulfilling the abovementioned criteria (Table 1). As a suggestion and in order to provide solid and timely genetic testing for patients fulfilling criteria A and C we propose upfront the performance of either whole exome sequencing (WES), whole genome sequencing (WGS) or large NGS panels complemented with the *in silico* CNV calling and/or laboratory analysis for CNVs, such as microarrays testing or MLPA (multiplex ligation amplification). At the moment WGS is however, not widely established in the clinical setting, but is being investigated as an alternative method in the Nordic countries. For the diagnostic procedure of Fanconi anemia, telomere biology disorders, Shwachman-Diamond syndrome, and Diamond-Blackfan anemia we refer to the respective international guidelines.[32–35]

If a potential germline variant has been detected in a somatic gene panel as part of the diagnostic work up for MN (see Criteria B above), further testing of extra-hematological tissue for the respective variant is highly recommended after obtaining the patient's consent. The patient may ideally be referred to genetic counseling for further information prior to testing of extra-hematological tissue.

Diagnostic algorithm

Please see Figure 1 for a proposal for a diagnostic algorithm. It should be highlighted that for specific syndromes such as *GATA2*-related disorders and *ANKDR26* even noncoding regulatory regions should be covered. Functional analyses may

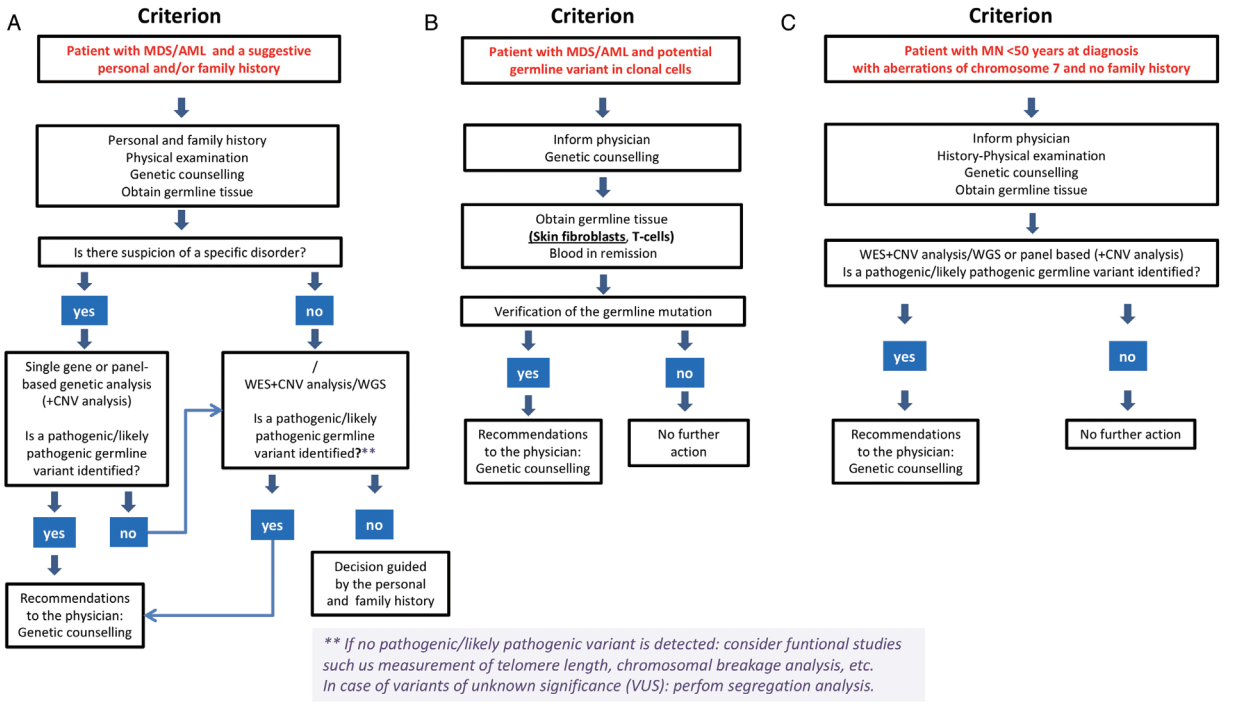

Figure 1. Diagnostic algorithm for the detection of germline variants predisposing to myeloid neoplasms (NMs). The algorithm is adjusted depending on the criterion which is fulfilled and aims in the detection of both single nucleotide variants (SNVs) and copy-number variations (CNVs). AML = acute myeloid leukemia; MDS = myelodyspastic syndrome; VUS = variant of unknown significance; WES = whole exome sequencing; WGS = whole genome sequencing.

be of value in Fanconi anemia, telomere biology disorders, and Diamond-Blackfan anemia especially when a variant of uncertain significance is detected.

Tissue for genetic testing

Regarding the tissue that should be analyzed we recommend fibroblasts obtained after skin biopsy, especially in cases fulfilling criteria A and C. In selected cases other alternatives such as blood in remission may also be appropriate. In cases included in criterion B a stepwise procedure with targeted analysis of sorted T-cells isolated from blood may be the first step. In case of confirmation of the pathogenic variant in the T-cells, a skin biopsy should also be performed. For carrier testing of healthy family members DNA from a whole blood sample can be used. Carrier testing, presymptomatic and predictive testing of healthy relatives require genetic counseling before and after genetic testing.

Genetic counseling

All patients with germline predisposition to MN should be offered genetic counseling. This also includes patients with a positive family history where the genetic pathogenic variant has not been identified. If the patient has not already received genetic counseling as part of the diagnostic procedures, it is important to offer counseling to ensure identification of family members at risk.

Surveillance of individuals with a germline predisposition to MDS or AML

The overall goal of surveillance and monitoring of patients with a germline predisposition to MDS and AML is to ensure intervention prior to development of high-risk disease. However, uncertainty regarding penetrance and age-adjusted risk of MDS and AML transformation makes it highly challenging to time such intervention correctly. In addition, germline disorders presenting as cytopenia, inherited bone marrow failure syndromes (IBMFS) or MDS may benefit more from surveillance as opposed to germline predisposition disorders presenting primarily as overt AML, especially if they occur in the context of a cancer syndrome with more than one organ involvement.

As very little evidence-based data exist on the efficacy and benefit of surveillance in individuals with germline predisposition to MDS and AML, published recommendations for surveillance are solely based on expert opinion.[26,28,38,44,50] For the classical IBMFS like Fanconi anemia, Shwachman-Diamond syndrome, Diamond-Blackfan anemia, and telomere biology disorders guidelines for surveillance already exist and should be followed when indicated in both children and adults.[32–35,51]

For whom is surveillance indicated

These recommendations for surveillance are intended for:

- Individuals with a deleterious or likely deleterious genetic variant associated with a germline predisposition regardless of clinical presentation.
- Individuals who fulfill the clinical diagnostic criteria for a myeloid neoplasm with a germline predisposition even if the pathogenic genetic variant is undetermined.

The recommendations do in general not apply to individuals with a variant of uncertain significance (VUS) in whom clinical diagnostic criteria for a specific predisposition disorder are absent.

If presymptomatic testing is impossible due to an unidentified pathogenic variant, and the family history is highly suggestive of hereditary MDS and AML, first-degree relatives to an affected

patient may in selected cases be considered for surveillance after genetic counseling.

Surveillance and follow-up at a hematology center with specialized expertise in disorders associated with germline predisposition to MDS/AML

All patients including asymptomatic carriers with a germline predisposition to MDS and AML should be referred to and subsequently followed by a hematological center with expertise in hereditary malignancies to ensure adequate monitoring and tailored treatment. The hematological centre/department is strongly recommended to collaborate closely with a clinical geneticists/medical genetic department with expertise in diagnosing and genetic counseling of hereditary haematologic disorders. If the patient has not already received genetic counseling as part of the diagnostic procedures, it is important to offer counseling to ensure identification and counseling of relatives at risk.

All patients should undergo physical examination at regular intervals, be educated about presentation and symptoms of MDS or AML and signs of other relevant conditions, and informed of limitations and benefits associated with surveillance.

Start of surveillance programs of asymptomatic mutation carriers must be individualized according to the typical age of myeloid neoplasm in the specific disorder and in the family. As examples *DDX41* associated MDS or AML presents in mid to late adulthood[13] whereas bone marrow failure syndromes typically present in childhood.

Work-up for patients with a germline predisposition to MDS or AML

Baseline

Initially all patients should have a diagnostic work-up for MN including a complete blood count (CBC) with manual differential, a bone marrow aspirate/biopsy with cytogenetic analysis and testing for somatic mutations using a myeloid gene panel according to the NMDSG guidelines. These investigations serve to exclude MDS or a manifest bone marrow failure disorder and as a baseline for subsequent comparison. It is important to recognize that asymptomatic carriers may present with varying blood counts ranging from normal CBC to mild or severe cytopenia. Also, carriers of *RUNX1*, *ANKRD26* and *ETV6* variants may have thrombocytopenia or bleeding tendency without thrombocytopenia at baseline.[20,52–57]

Follow-up

At the moment it is debatable how often routine follow-up investigations should be performed to monitor for disease progression.

Complete blood counts

The working group agreed that CBC should be repeated every 6 months particularly in high-risk patients i.e. those with pathogenic variants in *GATA2*, *RUNX1* or Fanconi complex genes.

If changes to abnormal values in blood counts develop, CBC should be repeated within a few weeks, other causes of cytopenia should be excluded, and a bone marrow biopsy/aspirate should be performed. Even a slight drop in thrombocytes just below normal range should warrant further investigations. See below.

Bone marrow aspirate/biopsy

Bone marrow aspirate/biopsy should not be routinely repeated if the CBC values are stable and other indications of progressive disease are absent. American guidelines in children and adults recommend annual bone marrow aspirate/biopsy in high-risk disorders,[38,50] because blood counts may not be sensitive enough as a marker for progression. However, it is the experience of the working group that asymptomatic carriers do not in general consent to annual bone marrow aspirate/biopsies and that the lack of compliance is not outweighed by the benefits of the procedure.

If deterioration of blood counts occurs on consecutive CBC, a bone marrow biopsy/aspirate is mandatory to examine for changes in bone marrow cellularity, dysplasia, blast percentage, clonal cytogenetic evolution, and somatic mutations.

Regarding clonal cytogenetic evolution it is important to note that some acquired aberrations like monosomy 7/del(7q)/der(1;7) and a complex karyotype are associated with high risk of malignant transformation, whereas others do not increase leukemic risk and can remain stable for long periods of time or even disappear. Isochromosome 7q and del(20q) in Shwachman-Diamond syndrome are examples of cytogenetic aberrations without associations to disease progression.[58,59]

Testing of peripheral blood for somatic mutations

There was consensus in the working group about the potential value of annual blood testing for somatic gene mutations with an NGS gene panel targeting myeloid genes with high coverage and reading depth. This recommendation is based on recent reports which highlight the emergence of clonal hematopoiesis associated with increased risk for the development of MDS or AML in hereditary conditions, mainly those related to germline *GATA2* and *RUNX1* mutations.[49,6,47,48] The frequency of testing for somatic mutations and the clinical implications are however, undefined in most other patients with germline predisposition to MDS or AML. Nevertheless, appearance in the blood of a new somatic mutation or a persistent or steep increase in variant allele frequency of an already existing clone should lead to further investigations including a bone marrow aspirate/biopsy in exactly the same way as emerging cytopenia (see "Complete blood count" above). The emergence of a clone should not solely be an indication for clinical action.[6] The gene, the VAF, the number of pathogenic variants as well as the dynamics over time should be taken into account. It should also be noted that the above-mentioned myeloid NGS panels are designed to include the great majority of potential somatic genomic aberrations that occur at the MDS or AML transformation, however they are restricted in their targets and a normal result cannot exclude somatic variant in genes not included in the panel-design.

For an overview of baseline and follow-up investigation of individuals with a germline predisposition see Table 2.

Management and surveillance of other organ dysfunction

Patients with germline predisposition to MDS/AML with risk of other organ dysfunction must be referred by the hematologist or the clinical/medical geneticist to a hereditary cancer clinic or to relevant medical disciplines/specialties to ensure screening for solid tumors and organ dysfunction depending on the specific risks and according to existing guidelines.[10,32–35]

Table 2

Follow-up of individuals with a germline predisposition to MDS/AML

	Baseline	Follow-up
Complete blood count (CBC)	YES	Every 6 months
Bone marrow aspirate/biopsy	YES	Only in case of change in CBC
NGS-myeloid gene panel	YES (bone marrow)	Once a year[a] (blood)
Control of other relevant organs	As indicated depending on the underlying condition	As indicated depending on the underlying condition

CBC = complete blood count; NGS = Next-generation sequencing.

[a] The emergence of a clone should not solely be an indication for action. The gene, the variant allele frequency (VAF), the number of pathogenic variants as well as the dynamics over time should be taken into account.

Referral to genetic counseling when family planning is relevant

Patients with germline predisposition to MDS/AML should be referred to genetic counseling when family planning is ongoing and preferably before pregnancy. At genetic counseling the couple should be informed about the risk in their offspring of inheriting the predisposition and options, if any, for prenatal diagnostics.

Considerations for allo-HSCT

As the hereditary myeloid disorders are highly heterogeneous it is outside the scope of the present guidelines to provide fully comprehensive recommendations regarding allo-HSCT. Instead this section contains general suggestions regarding indication, timing, donor selection, conditioning and follow-up of allo-HSCT for patients with "myeloid neoplasms with germline predisposition". Please note that especially the timing and the indication for allo-HSCT are based on expert-opinion due to the novelty and rarity of these syndromes.

Indication for allo-HSCT

All patients of a suitable age, who have developed MN on the basis of a genetic predisposition except those diagnosed with AML associated with germline variants in *CEBPA*, are potential candidates for allo-HSCT (see below). It should be highlighted that each case should be referred for discussion with an expert transplantation panel that may include international specialists in the field.

Timing of allo-HSCT

The exact diagnosis according to the WHO 2016 classification for myeloid neoplasms influences when allo-HSCT should be performed.

A: Myeloid neoplasm with germline predisposition without a pre-existing disorder or organ dysfunction

Patients with germline *CEBPA* mutations have no absolute indication for allo-HSCT in CR1 because they can experience long remissions after conventional chemotherapy,[60] but the risk of new leukemic clones is still high, therefore allo-HSCT may be considered at some time point in these patients. AML patients with germline *DDX41* mutations are often older and no specific recommendation regarding allo-HSCT can be made at the moment due to lack of data.[13,14]

B: Myeloid neoplasm with germline predisposition and pre-existing platelet disorder

For the time being there is no indication for allo-HSCT until the development of a MN.

C: Myeloid neoplasm with germline predisposition and other organ dysfunction

These syndromes show high risk of developing MN with a relatively early onset. Therefore, allo-HSCT could be considered before transformation occurs to prevent mortality due to other manifestations (*GATA2* related disorders).[10] In some individuals, even organ dysfunctions or life-threatening immunodeficiency may represent an indication for allo-HSCT. Timing of allo-HSCT in patients in this subgroup must be discussed with an expert panel that may even include international experts.

Donor selection

It is crucial to refrain from using an affected family member or an asymptomatic known carrier as donor in order to avoid graft failure and/or donor derived leukemia.[37] In families with an identified germline predisposing pathogenic variant we recommend genetic testing of any potential compatible donor in the absence of any signs/symptoms before HSCT. The genetic testing of a potential donor must be performed in respect of the individual's integrity and right "not to know" his or her carrier status. Genetic counseling is strongly recommended before testing if feasible. This procedure may take time, and in such cases a well-matched unrelated donor may be first choice to avoid harmful delay of the transplantation.

In families with a positive family history where the underlying genetic cause has not been identified an unrelated donor may be preferred even in the presence of phenotypically "healthy" matching family donors. However, donor selection must depend on availability and match.

Conditioning regimen

Individuals with IBMFS, like Fanconi anemia and telomere biology disorders, show an increased sensitivity to chemotherapy and specific dose-reduced conditioning regimens are recommended (for more information please see the respective treatment recommendations).[32,35,51]

Reduced intensity conditioning transplantation can also be used for patients with *GATA2* related syndromes before they have transformed because functional and numeric defects in the immune and hematopoietic stem cells will allow it.[61]

Follow up after allo-HSCT

Allo-HSCT increases the risk for secondary tumors, but many patients within the category "myeloid neoplasm with genetic predisposition and other organ dysfunction" have a specifically high risk so they should be monitored even more closely after allo-HSCT (Table 1).[32–35] See Table 1.

Concluding remarks

Germline predisposition to myeloid neoplasms is a new and rapidly emerging field, which significantly impacts therapy and care of involved patients and family members. Hence it is crucial that hematologists and clinical/medical geneticists have the basic knowledge to suspect a germline disorder. These Nordic guidelines are intended to support clinicians when dealing with patients with a potential germline predisposition to MDS or AML. The working group is aware that the criteria for genetic testing are conservative reflecting the fact that genetic testing is not widely available in all Nordic countries. However, this approach is aimed at identifying highly penetrant germline disorders, and as mentioned above the recommendations need to be updated and revised regularly.

At present we lack understanding on many biological and clinical aspects of the well-known and new germline disorders with increased risk of MDS or AML. Joint research projects and registries are urgently needed, and we strongly encourage hematologists and clinical/medical geneticists with special interest and expertise in the field to actively take part in such research activities.

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References

1. Huang KL, Mashl RJ, Wu Y, et al. Pathogenic Germline Variants in 10,389 Adult Cancers. *Cell.* 2018;173:355–370. e314.
2. Lu C, Xie M, Wendl MC, et al. Patterns and functional implications of rare germline variants across 12 cancer types. *Nat Commun.* 2015;6:10086.
3. Wartiovaara-Kautto U, Hirvonen EAM, Pitkanen E, et al. Germline alterations in a consecutive series of acute myeloid leukemia. *Leukemia.* 2018;32:2282–2285.
4. Drazer MW, Kadri S, Sukhanova M, et al. Prognostic tumor sequencing panels frequently identify germ line variants associated with hereditary hematopoietic malignancies. *Blood Adv.* 2018;2:146–150.
5. Tawana K, Drazer MW, Churpek JE. Universal genetic testing for inherited susceptibility in children and adults with myelodysplastic syndrome and acute myeloid leukemia: are we there yet? *Leukemia.* 2018;32:1482–1492.
6. Al Seraihi AF, Rio-Machin A, Tawana K, et al. GATA2 monoallelic expression underlies reduced penetrance in inherited GATA2-mutated MDS/AML. *Leukemia.* 2018.
7. Green CL, Tawana K, Hills RK, et al. GATA2 mutations in sporadic and familial acute myeloid leukaemia patients with CEBPA mutations. *Br J Haematol.* 2013;161:701–705.
8. Hsu AP, Johnson KD, Falcone EL, et al. GATA2 haploinsufficiency caused by mutations in a conserved intronic element leads to MonoMAC syndrome. *Blood.* 2013;121:3830–3837. S3831-3837.
9. Ostergaard P, Simpson MA, Connell FC, et al. Mutations in GATA2 cause primary lymphedema associated with a predisposition to acute myeloid leukemia (Emberger syndrome). *Nat Genet.* 2011;43:929–931.
10. Wlodarski MW, Collin M, Horwitz MS. GATA2 deficiency and related myeloid neoplasms. *Semin Hematol.* 2017;54:81–86.
11. Wlodarski MW, Hirabayashi S, Pastor V, et al. Prevalence, clinical characteristics, and prognosis of GATA2-related myelodysplastic syndromes in children and adolescents. *Blood.* 2016;127:1387–1397. quiz 1518.
12. Lewinsohn M, Brown AL, Weinel LM, et al. Novel germ line DDX41 mutations define families with a lower age of MDS/AML onset and lymphoid malignancies. *Blood.* 2016;127:1017–1023.
13. Polprasert C, Schulze I, Sekeres MA, et al. Inherited and somatic defects in DDX41 in myeloid neoplasms. *Cancer Cell.* 2015;27:658–670.
14. Tawana K, Fitzgibbon J. Inherited DDX41 mutations: 11 genes and counting. *Blood.* 2016;127:960–961.
15. Jeffries L, Shima H, Ji W, et al. A novel SAMD9 mutation causing MIRAGE syndrome: An expansion and review of phenotype, dysmorphology, and natural history. *Am J Med Genet A.* 2018;176:415–420.
16. Narumi S, Amano N, Ishii T, et al. SAMD9 mutations cause a novel multisystem disorder, MIRAGE syndrome, and are associated with loss of chromosome 7. *Nat Genet.* 2016;48:792–797.
17. Tesi B, Davidsson J, Voss M, et al. Cytopenia, predisposition to myelodysplastic syndrome, immunodeficiency, and neurological disease caused by gain-of-function SAMD9L mutations is frequently ameliorated by hematopoietic revertant mosaicism. *Blood.* 2016;128:
18. Moriyama T, Metzger ML, Wu G, et al. Germline genetic variation in ETV6 and risk of childhood acute lymphoblastic leukaemia: a systematic genetic study. *Lancet Oncol.* 2015;16:1659–1666.
19. Poggi M, Canault M, Favier M, et al. Germline variants in ETV6 underlie reduced platelet formation, platelet dysfunction and increased levels of circulating CD34+ progenitors. *Haematologica.* 2017;102:282–294.
20. Zhang MY, Churpek JE, Keel SB, et al. Germline ETV6 mutations in familial thrombocytopenia and hematologic malignancy. *Nat Genet.* 2015;47:180–185.
21. Arber DA, Orazi A, Hasserjian R, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. *Blood.* 2016;127:2391–2405.
22. Dohner H, Estey E, Grimwade D, et al. Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. *Blood.* 2017;129:424–447.
23. Guidelines. NCCN. Available at: https://www.nccn.org/professionals/physician_gls/pdf/mds.pdf. Accessed August 27 2019.
24. Brown AL, Churpek JE, Malcovati L, et al. Recognition of familial myeloid neoplasia in adults. *Semin Hematol.* 2017;54:60–68.
25. Churpek JE. Familial myelodysplastic syndrome/acute myeloid leukemia. *Best Pract Res Cl Ha.* 2017;30:287–289.
26. Churpek JE, Lorenz R, Nedumgottil S, et al. Proposal for the clinical detection and management of patients and their family members with familial myelodysplastic syndrome/acute leukemia predisposition syndromes. *Leuk Lymphoma.* 2013;54:28–35.
27. Churpek JE, Nickels E, Marquez R, et al. Identifying familial myelodysplastic/acute leukemia predisposition syndromes through hematopoietic stem cell transplantation donors with thrombocytopenia. *Blood.* 2012;120:5247–5249.
28. Furutani E, Shimamura A. Germline genetic predisposition to hematologic malignancy. *J Clin Oncol.* 2017;35:1018–1028.
29. Liew E, Owen C. Familial myelodysplastic syndromes: a review of the literature. *Haematologica.* 2011;96:1536–1542.
30. University of Chicago Hematopoietic Malignancies Cancer Risk T. How I diagnose and manage individuals at risk for inherited myeloid malignancies. *Blood.* 2016;128:1800–1813.
31. West AH, Godley LA, Churpek JE. Familial myelodysplastic syndrome/acute leukemia syndromes: a review and utility for translational investigations. *Ann Ny Acad Sci.* 2014;1310:111–118.
32. Fund. FAR. Available at: https://www.fanconi.org/images/uploads/other/FARF_Guidelines_Book_interior_lo-res.pdf. Accessed 2014.
33. Dror Y, Donadieu J, Koglmeier J, et al. Draft consensus guidelines for diagnosis and treatment of Shwachman-Diamond syndrome. *Ann N Y Acad Sci.* 2011;1242:40–55.
34. Vlachos A, Ball S, Dahl N, et al. Diagnosing and treating Diamond Blackfan anaemia: results of an international clinical consensus conference. *Br J Haematol.* 2008;142:859–876.
35. Teamtelomere. Available at: https://teamtelomere.org/wp-content/uploads/2018/07/DC-TBD-Diagnosis-And-Management-Guidelines.pdf. Accessed 2015.
36. Walsh MF, Chang VY, Kohlmann WK, et al. Recommendations for childhood cancer screening and surveillance in DNA repair disorders. *Clin Cancer Res.* 2017;23:e23–e31.
37. Galera P, Hsu AP, Wang W, et al. Donor-derived MDS/AML in families with germline GATA2 mutation. *Blood.* 2018;132:1994–1998.
38. Godley LA, Shimamura A. Genetic predisposition to hematologic malignancies: management and surveillance. *Blood.* 2017;130:424–432.
39. Rojek K, Nickels E, Neistadt B, et al. Identifying inherited and acquired genetic factors involved in poor stem cell mobilization and donor-derived malignancy. *Biol Blood Marrow Tr.* 2016;22:2100–2103.
40. Papaemmanuil E, Dohner H, Campbell PJ. Genomic classification in acute myeloid leukemia. *N Engl J Med.* 2016;375:900–901.
41. Papaemmanuil E, Gerstung M, Bullinger L, et al. Genomic classification and prognosis in acute myeloid leukemia. *N Engl J Med.* 2016;374:2209–2221.

42. Yannakou CK, Jones K, Ryland GL, et al. Incidental detection of germline variants of potential clinical significance by massively parallel sequencing in haematological malignancies. *J Clin Pathol.* 2018;71: 84–87.
43. Zhang JH, Walsh MF, Wu G, et al. Germline mutations in predisposition genes in pediatric cancer. *New Engl J Med.* 2015;373:2336–2346.
44. Babushok DV, Bessler M, Olson TS. Genetic predisposition to myelodysplastic syndrome and acute myeloid leukemia in children and young adults. *Leuk Lymphoma.* 2016;57:520–536.
45. Bluteau O, Sebert M, Leblanc T, et al. A landscape of germ line mutations in a cohort of inherited bone marrow failure patients. *Blood.* 2018;131:717–732.
46. Davidsson J, Puschmann A, Tedgard U, et al. SAMD9 and SAMD9L in inherited predisposition to ataxia, pancytopenia, and myeloid malignancies. *Leukemia.* 2018;32:1106–1115.
47. Churpek JE, Pyrtel K, Kanchi KL, et al. Genomic analysis of germ line and somatic variants in familial myelodysplasia/acute myeloid leukemia. *Blood.* 2015;126:2484–2490.
48. Wang X, Muramatsu H, Okuno Y, et al. GATA2 and secondary mutations in familial myelodysplastic syndromes and pediatric myeloid malignancies. *Haematologica.* 2015;100:e398–e401.
49. West RR, Hsu AP, Holland SM, et al. Acquired ASXL1 mutations are common in patients with inherited GATA2 mutations and correlate with myeloid transformation. *Haematologica.* 2014;99:276–281.
50. Porter CC, Druley TE, Erez A, et al. Recommendations for surveillance for children with leukemia-predisposing conditions. *Clin Cancer Res.* 2017;23:e14–e22.
51. Alter BP. Inherited bone marrow failure syndromes: considerations pre- and posttransplant. *Blood.* 2017;130:2257–2264.
52. Beri-Dexheimer M, Latger-Cannard V, Philippe C, et al. Clinical phenotype of germline RUNX1 haploinsufficiency: from point mutations to large genomic deletions. *Eur J Hum Genet.* 2008;16:1014–1018.
53. Owen CJ, Toze CL, Koochin A, et al. Five new pedigrees with inherited RUNX1 mutations causing familial platelet disorder with propensity to myeloid malignancy. *Blood.* 2008;112:4639–4645.
54. Preudhomme C, Renneville A, Bourdon V, et al. High frequency of RUNX1 biallelic alteration in acute myeloid leukemia secondary to familial platelet disorder. *Blood.* 2009;113:5583–5587.
55. Ripperger T, Tauscher M, Haase D, et al. Managing individuals with propensity to myeloid malignancies due to germline RUNX1 deficiency. *Haematologica.* 2011;96:1892–1894.
56. Topka S, Vijai J, Walsh MF, et al. Germline ETV6 mutations confer susceptibility to acute lymphoblastic leukemia and thrombocytopenia. *PLoS Genet.* 2015;11:e1005262.
57. Noris P, Favier R, Alessi MC, et al. ANKRD26-related thrombocytopenia and myeloid malignancies. *Blood.* 2013;122:1987–1989.
58. Minelli A, Maserati E, Nicolis E, et al. The isochromosome i(7)(q10) carrying c.258+2t>c mutation of the SBDS gene does not promote development of myeloid malignancies in patients with Shwachman syndrome. *Leukemia.* 2009;23:708–711.
59. Pressato B, Valli R, Marletta C, et al. Deletion of chromosome 20 in bone marrow of patients with Shwachman-Diamond syndrome, loss of the EIF6 gene and benign prognosis. *Br J Haematol.* 2012;157:503–505.
60. Tawana K, Wang J, Renneville A, et al. Disease evolution and outcomes in familial AML with germline CEBPA mutations. *Blood.* 2015;126:1214–1223.
61. Grossman J, Cuellar-Rodriguez J, Gea-Banacloche J, et al. Non-myeloablative allogeneic hematopoietic stem cell transplantation for GATA2 deficiency. *Biol Blood Marrow Transplant.* 2014;20:1940–1948.