Cancer Care Ontario

Consensus Pathology Recommendations for Complex Malignant Hematology

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Introduction

Current service models for Complex Malignant Hematology (CMH) in Ontario have been found to be variable and not well understood. Highly centralized services are available in some parts of the province and not in others, and referral patterns are not clearly articulated. Demand for CMH services has increased over time and is expected to continue to do so in the coming years. This increase brings with it additional stresses on the system as CMH patients require complex care with high resource utilization including Health Human Resources (HHR). As a result, Regional Cancer Programs in the province have identified pressures in meeting patient needs resulting in long wait times.

The Complex Malignant Hematology (CMH) & Hematopoietic Cell Therapy (HCT) Project was established in 2015 to drive improvements in three areas of CMH & HCT:

Provincial System: will have strong networks across the province that deliver care in a coordinated way and which meets patient needs;

Hospitals: will have the necessary infrastructure, health human resources (HHR) and funding to delivery high quality evidence-based care to all patients who need it; and

Patients: will have access to timely person-centred care that will ensure they have the best possible outcomes.

To support the provincial CMH initiative a number of working groups were established, including a Models of Care Working Group, Leukemia Provincial Planning Working group, as well as a Pathology CMH Working Group

The Pathology CMH Working Group was comprised of a multidisciplinary group of health care providers including Pathologists, Geneticists, and Hematologists. The purpose of the Pathology CMH working group was to provide advice and develop recommendations for laboratory testing best practices for Acute Myeloid Leukemia (AML), Acute Lymphoblastic Leukemia (ALL), Myelodysplastic Syndromes (MDS), High-Grade Lymphoma, and Aplastic Anemia. The use of Minimal Residual Disease (MRD) testing was considered within the scope of this working group.

The established group worked to develop recommendations to standardize the diagnosis of the identified diseases, including the types, timing, and frequency of laboratory testing. Best practice recommendations are based on available evidence or consensus. The document also identifies additional topics for future consideration.

Preamble

- Biomarker testing is essential for the management of hematologic malignancies. Biomarkers can be assessed by a variety of techniques including, but not limited to, immunohistochemistry (IHC), in-situ hybridization (ISH and FISH), G-band karyotyping, array comparative genomic hybridization (aCHG) and a variety of other techniques including those using polymerase chain reaction (PCR) and/or nucleic acid sequencing.
- Technologies to identify biomarkers continue to change and laboratory infrastructure can be variable, making it important that each laboratory determine which technologies and laboratory processes are best suited for assessment of each biomarker to meet clinical need in a cost-effective manner. It is the responsibility of the laboratory in conjunction with established Communities of Practice to ensure that minimum established biomarkers and parameters of test performance are met, regardless of the methods or platforms chosen.
- The diagnosis of hematologic malignancies involves evaluation for multiple biomarkers, some of which need to be assessed rapidly, to ensure identification of patients requiring immediate administration of life-saving therapy. Other assays can be performed less urgently during the initial evaluation phase to fully characterize the disease, determine patient prognosis and identify patients who may require stem cell transplant. Biomarkers may be tested using different methods depending on a number of factors, including clinical need and timing.
- As the number of biomarkers expand, newer technologies such as next generation sequencing (NGS) that allow for simultaneous analysis of multiple biomarkers are amenable for the management of hematologic malignancies. As these recommendations focus on necessary biomarkers and required clinical timing, rather than technologies, it is important to recognize that the implementation of these recommendations will require increased access to technologies that streamline biomarker testing.
- Communities of practice need to be established with all laboratory sites including, hematologists, hematopathologists and geneticists to ensure that the required biomarkers for patient management reflect clinical practice and are consistent throughout the province.
- The investigation of hereditary disorders was considered to be outside of the scope of this work, and specific testing for hereditary causes of hematologic diseases was not included in the recommendations. This has been identified as a topic for future consideration.
- For the purposes of this work, high-grade lymphoma was defined by the Specialized Services Oversight Program. The work of this group focused on specific biomarkers that need to be available for these high-grade lymphomas.

Acute Myeloid Leukemia

Classification

Acute Myeloid Leukemia (AML) should be classified per the 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia *(Appendix A – Table 1)*.

Diagnostic Workup

- Upon suspicion, all patients should receive:
  - CBC & Blood Smear
  - Bone Marrow – aspirate / biopsy
  - Morphology
  - Flow Cytometry
- Following initial confirmation / suspicion of AML, patients should receive the following:
  - Cytogenetics/ karyotyping (G-band/ FISH Analysis)
  - Evaluation of genomic biomarkers for any or all of diagnostic sub-classification, prognosis and therapeutic guidance *(Appendix B – Table 5)*
  - Classification as per WHO diagnostic category *(Appendix A – Table 1)*
  - Preparation for HLA Typing when appropriate

Turn-around-times (TAT - Calendar Days)

- Initial diagnosis of AML - (within 48 hours)
- *PML/RARA* - (within 24 hours)
- *BCR-ABL1* - (within 5 days)
- *FLT3(ITD), CEBPA, NPM1* – (within 5 days)
- G-band karyotype / FISH analysis – (within 14 days)
- Complete genomic characterization – (within 21 days)
- HLA Typing - (within 14 days)

Minimal Residual Disease (MRD) Testing and Quantitative Monitoring in AML

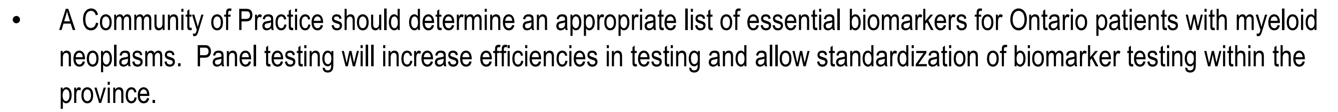
- AML with specific biomarkers (*PML/RARA*, *RUNX1/RUNX1T1*, *CBFB/MYH11*, *BCR-ABL1*, *NPM1*) or other selected cytogenetic alterations
  - Timing determined by treatment protocols and ongoing follow-up (Appendix C)
- Further investigation is needed to determine the role of testing for additional biomarkers for MRD in AML

Topics Requiring Further Investigation / Evidence

- mRNA expression analysis and miRNA analysis is not required, but there is emerging evidence that these markers may be useful for prognosis and management of AML in the future *(Appendix B – Table 6)*

AML – Key Summary

- Genomic testing is required for AML diagnosis, prognosis and therapeutic management decisions.
- Information about selected biomarkers is required rapidly for important, early management decisions.
- MRD investigation has a role in the current management of AML patients.
- The list of useful genomic biomarkers for management of AML is large and continues to evolve. It should be re-visited on an annual basis.



- A Community of Practice should determine an appropriate list of essential biomarkers for Ontario patients with myeloid neoplasms. Panel testing will increase efficiencies in testing and allow standardization of biomarker testing within the province.

Acute Lymphoblastic Leukemia

Classification

Acute Lymphoblastic Leukemia (ALL) should be classified per the 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia *(Appendix A – Table 2).*

Diagnostic Workup

- Upon suspicion, all patients should receive:
    - CBC and blood smear
    - Bone Marrow – aspirate / biopsy
    - Morphology
    - Flow Cytometry
- Following initial confirmation / suspicion of ALL, patients should receive the following:
    - Cytogenetics/ karyotyping (G-banding/ FISH Analysis)
    - Evaluation of genomic biomarkers for diagnostic sub-classification per current WHO diagnostic categories *(Appendix A – Table 2)*.
    - Preparation for HLA Typing when appropriate
    - B-cell and T-cell gene rearrangements (when appropriate)

Turn-around-time (TAT - Calendar Days)

- Diagnosis of ALL (without most genomic biomarkers for sub-classification) – (within 48 hours)
- Analysis of *BCR-ABL1* – (within 5 days).
- Cytogenetic/G-band karyotype, FISH analysis – (within 14 days)
- Tumor-specific immunoglobulin or T-cell receptor gene rearrangements for clonotypic analysis – (within 14 days when required for diagnosis; within 28 days when required for MRD testing alone)
- HLA Typing – (within 14 days)

Minimal Residual Disease Testing (MRD)

- The use of MRD testing for ALL is required
- Analysis of tumor/patient-specific immunoglobulin or T-cell receptor gene rearrangements
- *BCR-ABL1* monitoring every 3 months (in *BCR-ABL1* positive cases).
    - Monitoring frequency for other genes continues to evolve and will require further investigation.
- Other biomarkers and evaluation methods for MRD testing in ALL may need to be established

ALL – Key Summary

- Genomic testing is required for ALL diagnosis (which currently includes prognostic and therapeutic information).
- Selected biomarkers (*BCR-ABL1*) are required rapidly for important, early management decisions.
- MRD has a role in the current management of ALL patients.
- The list of useful genomic biomarkers for management of ALL is large and continues to evolve. It should be re-visited on an annual basis.
- A Community of Practice should determine an appropriate list of essential biomarkers for Ontario patients with ALL.

Myelodysplastic Syndromes

Classification

- Myelodysplastic Syndromes (MDS) should be classified per the 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia *(See Appendix A – Table 3).*

Diagnostic Workup

- Upon suspicion, all patients should receive:
    - CBC and blood smear
    - Bone Marrow – aspirate / biopsy
    - Morphology
    - Flow Cytometry (optional)
- With morphologic suspicion / diagnosis of MDS patients should receive the following:
    - Cytogenetics (G-banding karyotype)
    - Evaluation of additional genomic biomarkers as required for any or all of diagnostic sub-classification, prognosis and therapeutic guidance *(Appendix A – Table 3 and Appendix B – Table 5)*
    - Preparation for HLA Typing when appropriate

Turn-around-times (TAT - Calendar Days)

- Initial diagnosis of MDS (if it can be made without genomic testing) – (within 48 hours)
- Cytogenetic/G-banding karyotype, FISH – (within 14 days)
- Complete genomic characterization – (within 21 days)

MDS – Key Summary

- Genomic testing is required for MDS diagnosis, prognosis and therapeutic management decisions.
- The list of useful genomic biomarkers for management of MDS is large and continues to evolve. It should be re-visited on an annual basis.
- Community of practice should determine an appropriate list of essential biomarkers for Ontario patients with MDS.

Aplastic Anemia

The working group focused on investigations for patients in whom a diagnosis of aplastic anemia is established and for whom stem cell transplant is being considered. The complete workup of pancytopenia is beyond the scope of the working group.

The investigation and care of patients with aplastic anemia requires evaluation of the emergence of myelodysplasia (MDS), leukemia (AML), or paroxysmal nocturnal hemoglobinuria (PNH). Should MDS / AML be suspected, patients should be investigated as outlined in the recommendations for MDS/ AML.

The suggested minimal diagnostic workup for patients with aplastic anemia is outlined in *Appendix D – Table 8*.

The selection of patients for transplant involves a combination of clinical symptoms, response to therapy and peripheral blood counts. Key recommendations for patients being considered for stem cell transplant can be found in *Appendix E*.

Key Message

- Patients with Aplastic Anemia need to have MDS / AML excluded and be monitored for the development of MDS / AML, and PNH.
- Patients considered for stem cell transplant should have HLA testing performed as soon as transplant is being considered.
- Aplastic Anemia patients may develop myeloid neoplasm-associated mutations seen in MDS and AML. Some biomarkers have increased frequency compared to MDS/AML include *PIGA*, *BCOR*, and *BCORL1* (Yoshizato *et al*, 2015); patients with suspected myeloid neoplasms should be investigated as outlined for AML/MDS.
- Routine extensive biomarker testing is not indicated for Aplastic Anemia at this time, unless suspicion arises for the development of a myeloid neoplasm.

High-Grade Lymphoma

The complete workup of lymphoma is beyond the scope of the working group. The working group identified key biomarkers that should be assessed to determine the prognosis of patients with high-grade lymphoma and to identify patients who may be considered for stem cell transplant. Stem cell transplants are also indicated in other types of non-high grade lymphomas as part of ongoing treatment that may be out of scope for this document. These include but are not limited to; follicular lymphoma, mantle cell lymphoma, refractory Hodgkin's lymphoma multiple myeloma *(Appendix F).*

The assessment of specific molecular markers tied to investigational therapy (PD-L1 / tyrosine kinase analysis) is beyond the scope of this working group. It is recognized that emerging therapies for lymphoma and other diseases may include specific biomarker investigations which would need to be included in the diagnostic workup as these therapies move into practice.

Classification

Lymphomas should be classified as per the 2016 revision to the World Health Organization classification of lymphoid neoplasms *(Appendix A – Table 4).*

Diagnostic Workup

- *BCL2* (*Bcl-2*), *BCL6* (*Bcl-6*), *MYC* (*c-Myc*), Epstein Barr Virus (*EBV)*
  - Requires immunohistochemical (IHC) / *in situ* hybridization (ISH) analysis and molecular / cytogenetics testing
- Specific molecular testing may be required for certain entities within the WHO 2016 classification, but these are for sub-classification of the disease and not required for all high-grade lymphoma cases
- HLA typing (where appropriate)

Turn-around-times (TAT - Calendar Days)

- IHC analysis – (within 24 hours)
- *DNA rearrangement analysis – (within 5 days)*
- Other markers – (within 14 days)

Topics Requiring Further Investigation / Evidence

- RNA expression analysis
- 11q aberration for Burkitt–like lymphoma
- Minimal Residual Disease Testing (MRD) for High Grade Lymphoma

Appendix A: WHO Classifications of AML, ALL, MDS, and Lymphoid Neoplasms

Table 1: WHO Classification of Acute Myeloid Leukemia (AML) and related neoplasms (Arber et al., 2016)	
Acute myeloid leukemia with recurrent genetic abnormalities	• AML with t(8;21)(q22;q22.1);*RUNX1-RUNX1T1* • AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22);*CBFB-MYH11* • APL with *PML-RARA* • AML with t(9;11)(p21.3;q23.3);*MLLT3-KMT2A* • AML with t(6;9)(p23;q34.1);*DEK-NUP214* • AML with inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2); *GATA2, MECOM* • AML (megakaryoblastic) with t(1;22)(p13.3;q13.3);*RBM15-MKL1* • Provisional entity: AML with *BCR-ABL1* • AML with mutated *NPM1* • AML with biallelic mutations of *CEBPA* • *Provisional entity: AML with mutated RUNX1*
AML with myelodysplasia-related changes	
Therapy-related myeloid neoplasms	
Acute myeloid leukemia, Not otherwise specified (NOS)	• AML with minimal differentiation • AML without maturation • AML with maturation • Acute myelomonocytic leukemia • Acute monoblastic/monocytic leukemia • Pure erythroid leukemia • Acute megakaryoblastic leukemia • Acute basophilic leukemia • Acute panmyelosis with myelofibrosis
Myeloid Sarcoma	
Myeloid proliferations related to Down syndrome	• Transient abnormal myelopoiesis (TAM)

	• Myeloid leukemia associated with Down Syndrome

Table 2: WHO Classification of Acute Lymphoblastic Leukemias (ALL) (Arber et al., 2016)	
B lymphoblastic leukemia/lymphoma	• B-lymphoblastic leukemia/lymphoma, NOS • B-lymphoblastic leukemia/lymphoma with recurrent genetic abnormalities • B-lymphoblastic leukemia/lymphoma with t(9;22)(q34.1;q11.2);*BCR-ABL1* • B-lymphoblastic leukemia/lymphoma with t(v;11q23.3);*KMT2A* rearranged • B-lymphoblastic leukemia/lymphoma with t(12;21)(p13.2;q22.1); *ETV6-RUNX1* • B-lymphoblastic leukemia/lymphoma with hyperdiploidy • B-lymphoblastic leukemia/lymphoma with hypodiploidy • B-lymphoblastic leukemia/lymphoma with t(5;14)(q31.1;q32.3) *IL3-IGH* • B-lymphoblastic leukemia/lymphoma with t(1;19)(q23;p13.3);*TCF3-PBX1* • *Provisional entity: B-lymphoblastic leukemia/lymphoma, BCR-ABL1–like* • *Provisional entity: B-lymphoblastic leukemia/lymphoma with iAMP21*
T-lymphoblastic leukemia/lymphoma	• *Provisional entity: Early T-cell precursor lymphoblastic leukemia* • *Provisional entity: Natural killer (NK) cell lymphoblastic leukemia/lymphoma*

Table 3: WHO Classification of Myelodysplastic Syndromes and Myelodysplastic/ Myeloproliferative Neoplasms (Arber et al., 2016)	
Myelodysplastic/ myeloproliferative neoplasms (MDS/MPN)	• Chronic myelomonocytic leukemia (CMML) • Atypical chronic myeloid leukemia (aCML), *BCR-ABL1*$^{-}$ • Juvenile myelomonocytic leukemia (JMML) • MDS/MPN with ring sideroblasts and thrombocytosis (MDS/MPN-RS-T) • MDS/MPN, unclassifiable
Myelodysplastic syndromes (MDS)	• MDS with single lineage dysplasia • MDS with ring sideroblasts (MDS-RS) • MDS-RS and single lineage dysplasia

	• MDS-RS and multilineage dysplasia • MDS with multilineage dysplasia • MDS with excess blasts • MDS with isolated del(5q) • MDS, unclassifiable • *Provisional entity: Refractory cytopenia of childhood* • Myeloid neoplasms with germ line predisposition

Table 4: 2016 revision of the World Health Organization classification of lymphoid neoplasms (Swerdlow et al., 2016)
Mature B-cell neoplasms
Chronic lymphocytic leukemia/small lymphocytic lymphoma
Monoclonal B-cell lymphocytosis*
B-cell prolymphocytic leukemia
Splenic marginal zone lymphoma
Hairy cell leukemia
Splenic B-cell lymphoma/leukemia, unclassifiable
Splenic diffuse red pulp small B-cell lymphoma
Hairy cell leukemia-variant
Lymphoplasmacytic lymphoma
Waldenström macroglobulinemia
Monoclonal gammopathy of undetermined significance (MGUS), IgM*
μ heavy-chain disease
ɣ heavy-chain disease
α heavy-chain disease
Monoclonal gammopathy of undetermined significance (MGUS), IgG/A*
Plasma cell myeloma
Solitary plasmacytoma of bone
Extraosseous plasmacytoma
Monoclonal immunoglobulin deposition diseases*
Extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma)
Nodal marginal zone lymphoma
Pediatric nodal marginal zone lymphoma
Follicular lymphoma
In situ follicular neoplasia*
Duodenal-type follicular lymphoma*
Pediatric-type follicular lymphoma*
*Large B-cell lymphoma with IRF4 rearrangement**
Primary cutaneous follicle center lymphoma
Mantle cell lymphoma

In situ mantle cell neoplasia*
Diffuse large B-cell lymphoma (DLBCL), NOS
Germinal center B-cell type*
Activated B-cell type*
T-cell/histiocyte-rich large B-cell lymphoma
Primary DLBCL of the central nervous system (CNS)
Primary cutaneous DLBCL, leg type
EBV+ DLBCL, NOS*
*EBV+ mucocutaneous ulcer**
DLBCL associated with chronic inflammation
Lymphomatoid granulomatosis
Primary mediastinal (thymic) large B-cell lymphoma
Intravascular large B-cell lymphoma
ALK+ large B-cell lymphoma
Plasmablastic lymphoma
Primary effusion lymphoma
HHV8+ DLBCL, NOS*
Burkitt lymphoma
*Burkitt-like lymphoma with 11q aberration**
High-grade B-cell lymphoma, with *MYC* and *BCL2* and/or *BCL6* rearrangements*
High-grade B-cell lymphoma, NOS*
B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and classical Hodgkin lymphoma
Mature T and NK neoplasms
T-cell prolymphocytic leukemia
T-cell large granular lymphocytic leukemia
Chronic lymphoproliferative disorder of NK cells
Aggressive NK-cell leukemia
Systemic EBV+ T-cell lymphoma of childhood*
Hydroa vacciniforme–like lymphoproliferative disorder*
Adult T-cell leukemia/lymphoma
Extranodal NK-/T-cell lymphoma, nasal type
Enteropathy-associated T-cell lymphoma
Monomorphic epitheliotropic intestinal T-cell lymphoma*
*Indolent T-cell lymphoproliferative disorder of the GI tract**
Hepatosplenic T-cell lymphoma
Subcutaneous panniculitis-like T-cell lymphoma
Mycosis fungoides
Sézary syndrome
Primary cutaneous CD30+ T-cell lymphoproliferative disorders
Lymphomatoid papulosis
Primary cutaneous anaplastic large cell lymphoma
Primary cutaneous γδ T-cell lymphoma
Primary cutaneous CD8+ aggressive epidermotropic cytotoxic T-cell lymphoma
*Primary cutaneous acral CD8+ T-cell lymphoma**
*Primary cutaneous CD4+ small/medium T-cell lymphoproliferative disorder**
Peripheral T-cell lymphoma, NOS
Angioimmunoblastic T-cell lymphoma

*Follicular T-cell lymphoma**
*Nodal peripheral T-cell lymphoma with TFH phenotype**
Anaplastic large-cell lymphoma, ALK+
Anaplastic large-cell lymphoma, ALK-*
*Breast implant–associated anaplastic large-cell lymphoma**
Hodgkin lymphoma
Nodular lymphocyte predominant Hodgkin lymphoma
Classical Hodgkin lymphoma
Nodular sclerosis classical Hodgkin lymphoma
Lymphocyte-rich classical Hodgkin lymphoma
Mixed cellularity classical Hodgkin lymphoma
Lymphocyte-depleted classical Hodgkin lymphoma
Posttransplant lymphoproliferative disorders (PTLD)
Plasmacytic hyperplasia PTLD
Infectious mononucleosis PTLD
Florid follicular hyperplasia PTLD*
Polymorphic PTLD
Monomorphic PTLD (B- and T-/NK-cell types)
Classical Hodgkin lymphoma PTLD
Histiocytic and dendritic cell neoplasms
Histiocytic sarcoma
Langerhans cell histiocytosis
Langerhans cell sarcoma
Indeterminate dendritic cell tumor
Interdigitating dendritic cell sarcoma
Follicular dendritic cell sarcoma
Fibroblastic reticular cell tumor
Disseminated juvenile xanthogranuloma
Erdheim-Chester disease*

Provisional entities are listed in italics.
*Changes from the 2008 classification.

Appendix B: Initial Gene Lists for Myeloid Neoplasms

Table 5: Initial Myeloid Gene List for AML / MDS NGS panel	
ASXL1	*MPL*
BCOR	*NPM1**
BRAF	*NRAS*
CALR	*RAD21*
*CEBPA**	*RUNX1*
DNMT3A	*SF3B1*
EZH2	*SRSF2*
*FLT3 (ITD)**	*STAG2*
IDH1	*TET2*
IDH2	*TP53*
JAK2	*U2AF1*
KIT	*WT1*
KMT2A (PTD)	*ZRSR2*

* Analysis required within 5 days or less

Table 6: Potential Gene List for mRNA Expression Analysis in AML	
BAALC	microRNA (miR-155 / 181a / 3151)
DNMT3B	*MN1*
ERG	*SPARC*
MECOM/EVI1	

Appendix C:

Table 7: Minimal Residual Disease Monitoring Schedule for AML and ALL following chemotherapy and allogeneic transplant*					
AML *With specific tx or NPM1*	**At Diagnosis**	**At 6 weeks**	**Every 3 months for years 1&2**	**Every 6 months for year 3**	**Once/year at year 4 then only if required due to clinical symptoms**
ALL *IgH, TCR or specific tx*	**At Diagnosis**	**At 6 weeks**	**Every 3 months for years 1 - 4**	**Once/year at year 5 then only if required due to clinical symptoms**	

*Monitoring can be more frequent if clinical symptoms/response justify it.

Appendix D:

Table 8: Summarized Diagnosis and Further Investigation of Aplastic Anemia (Killick et al., 2015)	
Test	**Key Changes**
1. Full blood count	Pancytopenia. Usually the haemoglobin concentration and neutrophil and platelet counts are uniformly depressed. In the early stages, isolated cytopenia, particularly thrombocytopenia, may occur. Lymphocyte counts are usually preserved. Presence of monocytopenia needs further investigation to exclude hairy cell leukaemia or inherited bone marrow failure due to *GATA2* mutation (Emberger/ MonoMac syndrome, see section on inherited AA)
2. Reticulocyte count	Reticulocytopenia; automated reticulocyte counting will over-estimate the count compared with the levels set in the Camitta criteria (Camitta, 1984) for defining disease severity, which were defined on manual counts. This criterion has now been modified from manual percentages to absolute reticulocyte levels <60 9 109/l as assessed by automated technologies (Rovo et al, 2013)
3. Blood film examination	Frequent macrocytosis and anisopoikilocytosis. Neutrophils may show toxic granulation. Platelets are mainly small in size. Exclude presence of dysplastic neutrophils, abnormal platelets, blasts and other abnormal cells, such as 'hairy' cells
4. HbF%	HbF; measure pre-transfusion in children – important prognostic factor in children. Note that the level is often elevated in constitutional syndromes
5. Peripheral blood chromosomal breakage analysis: diepoxybutane test (DEB Test)	For possible FA if patient aged <50 years, but it would also be indicated to screen older patients if FA is clinically suspected. It is difficult to set an upper age limit for FA screening, as anecdotal cases have been diagnosed in the fifth decade (unpublished observations). Screen all patients who are transplant candidates and siblings of FA patients
6. Flow cytometry for GPI-anchored proteins to detect PNH clone (6- colour methodology including FLAER)	See AA and PNH section for full description
7. Vitamin B12 and folate	Documented vitamin B12 or folate deficiency should be corrected before a final diagnosis of AA is confirmed. Bone marrow aplasia due to vitamin deficiency is exceedingly rare
8. Liver function tests	Liver function tests should be performed to detect antecedent/on-going hepatitis

9. Viral studies: hepatitis A/B/C, EBV, CMV, HIV and Parvovirus B19	AA due to hepatitis is rare, it usually occurs 2–3 months after an acute episode of hepatitis and is more common in young males (Brown et al, 1997). In post-hepatic AA the serology is often negative for the known hepatitis viruses. CMV should be assessed if SCT is being considered. HIV more commonly causes isolated cytopenias but is a very rare cause of AA (Wolf et al, 2007; Hapgood et al, 2013). Likewise, parvovirus B19 is more usually associated with pure red aplasia but has been reported with AA (Mishra et al, 2005)
10. Anti-nuclear antibody and antidouble stranded DNA	Pancytopenia in systemic lupus erythematosus may (i) be autoimmune with a cellular bone marrow (ii) associated with myelofibrosis or rarely (iii) with a hypocellular marrow
11. Chest X-ray and other radiology	Useful at presentation to exclude infection and for comparison with subsequent films. X-rays of the hands, forearms and feet may be indicated if an IBMFS is suspected. High resolution CT scan of the chest is indicated for suspected DC or constitutional *RUNX1* bone marrow failure syndrome
12. Abdominal ultrasound scan and echocardiogram	An enlarged spleen and/or lymph nodes raise the possibility of a malignant haematological disorder as the cause of the pancytopenia. In younger patients, abnormal or anatomically displaced kidneys are features of FA
13. Emerging diagnostic tests: the following are not currently routine diagnostic tests, but are likely to be so within the next few years	
• Peripheral blood leucocyte telomere length:	Useful for disease screening for telomere gene mutations in classic DC; less specific in adult onset AA with *TERC/TERT* mutations; short telomeres may also occur in acquired AA with reduced stem cell reserve (Townsley et al, 2014)
• Next generation sequencing, gene panels for:	• Telomere gene complex mutations • Other IBMFS • Acquired somatic mutations, typical of myeloid malignancies, to help distinguish AA from hypocellular MDS and for early detection of clonal evolution to MDS/AML (Kulasekararaj et al, 2014)
• Single nucleotide polymorphism array karyotyping	Whole genome scanning to detect unbalanced chromosomal defects (Afable et al, 2011a)

HbF, fetal haemoglobin; GPI, glycerophosphatidylinositol; AA, aplastic anaemia; PNH, paroxysmal nocturnal haemoglobinuria; FLAER, fluorescent aerolysin; EBV, Epstein–Barr virus; CMV, cytomegalovirus; HIV, human immunodeficiency virus; SCT, stem cell transplantation; IBMFS, inherited bone marrow failure syndromes; MDS, myelodysplastic syndrome; AML, acute myeloid leukaemia; CT, computerized tomography; DC, dyskeratosis congenita; FA, Fanconi anaemia.

Appendix E:

Key Recommendations for Haemopoietic Stem Cell Transplantation (Adapted from Killick et al., 2015)

All patients being considered for HSCT should be evaluated in a multi-disciplinary team setting, and consideration should be given to discussion of the case with a centre that has expertise in AA regarding the indications for HSCT and the choice of conditioning regimen.

- To inform the multi-disciplinary team decision-making regarding HSCT:
  - All patients who are potential HSCT candidates should undergo human leucocyte antigen (HLA) typing as soon as transplant is considered for patient management.
  - A careful reassessment should be made to confirm the precise diagnosis and exclude clonal evolution to myelodysplastic syndrome (MDS) or paroxysmal nocturnal haemoglobinuria (PNH), as this will influence the choice of conditioning.
  - It is also vital not to miss constitutional AA so as to avoid (i) serious (and potentially lethal) toxicity from the transplant and (ii) inappropriate selection of a sibling donor.

Appendix F:

Lymphoma indications for Auto Peripheral Blood Stem Cell Transplant (Ferreri, 2008)

- Autologous stem cell transplantation is recommended for chemo-sensitive patients with H L who are refractory to or have relapsed after primary chemotherapy.
- Autologous stem cell transplantation is recommended for eligible younger patients (under age 70 years) with newly diagnosed MM (primary amyloid)
- Tandem (double) autologous stem cell transplantation is an option for patients who obtain less than a complete (or near) response to the first autologous transplant
- Repeat autologous transplantation is an option for patients with MM who relapse after a long remission [> 2 years] to a single autologous transplant.
- Autologous stem cell transplantation is recommended for chemo-sensitive patients with B or T NHL refractory to or relapsed after primary therapy.
- Autologous transplantation is an option for selected patients with poor prognosis FL that progresses after second-line therapy (under debate)
- Autologous transplantation for selected patients with Burkitt's lymphoma beyond first remission
- Autologous stem cell transplantation for eligible patients with MCL in first remission.
- Autologous stem cell transplantation (single or tandem) is a treatment option for patients with gonadal or retroperitoneal germ cell tumours refractory to or relapsed after cisplatin-based chemotherapy.
- Autologous transplantation for selected patients with primary CNS NHL beyond first remission/consolidation (Thiotepa, Cyclo, Busulfan)

Glossary

AA – Aplastic Anemia

ALL – Acute Lymphoblastic Leukemia

AML – Acute Myeloid Leukemia

CBC – Complete Blood Count

CCO – Cancer Care Ontario

CMH – Complex Malignant Hematology

FISH – Fluorescence In-Situ Hybridization

HCT – Haematopoietic Cell Transplant

HCT – Hematopoietic Cell Therapy

HHR – Health Human Resources

HLA – Human Leukocyte Antigen

HSCT – Haemopoietic Stem Cell Transplantation

MDS – Myelodysplastic Syndromes

MRD – Minimal Residual Disease

NGS – Next Generation Sequencing

PCR – Polymerase Chain Reaction

TAT – Turn Around Time

WHO – World Health Organization

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