Detection of GPI-A deficient cells in Paroxysmal Nocturnal Hemoglobinuria (PNH) and Bone Marrow Failure Syndromes (BMFS) by Flow Cytometry

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Disclosure
I have no potential conflict of interest

Initial viral insult
Proinflammatory environment
Immune mediated bone marrow injury

Heterogeneous group
Overlapping diseases
Propensity to develop MDS/AML
Acquired forms ≥80%
Idiopathic forms >75%

Initial viral insult
Proinflammatory environment
Immune mediated bone marrow injury

Small PNH clone
Minor clone (<1%)/cells with PNH phenotype (<0.1%)

Clinical utility of testing for GPI-A deficient clones in PNH

Clinical utility of testing for GPI-A deficient clones in BMFS

Diagnostic Workflow for iBMFS

Presence of GPI deficient clone: high NPV for BMFS
Presence of GPI-deficient clone in patients ≥60 years have germline mutations

Clinical utility of testing for GPI-A deficient clones in BMFS

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Clinical utility of testing for GPI-A deficient clones in BMFS
Diagnostic workflow for AA

- FBC: pancytopenia, anaemia with reticulocytopenia, macrocytosis....
- BM aspirate and trephine biopsy
  - aplastic bone marrow, without dysplasia and blast infiltration

Mutation analysis

- 20 events for reproducible detection
- Less favorable: MDS

More favorable:

- Adverse <5% blasts:
  - PNH clone
  - FISH
- Favorable:
  - AA
  - 35%

Other conditions:

- MDS associated mutations also occur in healthy older individuals
- PNH

GPI anchor deficiency evaluation by Flow Cytometry

- Evidence of bone marrow failure, including severe hypo/reticulocytopenia, macrocytosis, or hypoplastic anemia.

Importance of high sensitivity assay (> 0.01%)

Diagnostic workflow for h-MDS

- FBC: pancytopenia, anaemia with reticulocytopenia, macrocytosis....
- BM aspirate and trephine biopsy
  - Hypoplastic bone marrow (h-MDS): morphological evidence of dysplasia

Cytogenetics / FISH

- 4% MDS (4q, 11q, 7q, 8q, 20q, ...)
- Advanced diagnostic clone does not imply the dg. MDS

Mutation analysis

- 20% associated with myeloid genes typically detected in MDS

Other conditions:

- PNH

GPI anchor deficiency evaluation by Flow Cytometry

- Evidence of bone marrow failure, including severe hypo/reticulocytopenia, macrocytosis, or hypoplastic anemia.

Diagnostic workflow for Acquired Aplastic Anaemia

- Patients with AA should be screened for PNH at the diagnosis of AA.

ICCS/ESCCA Guidelines 2018

- PROVIDE VALIDATED APPROACHES meeting criteria for high:
  - Accuracy / Thruemness - ICA, ILC
  - Clinical specificity / TN/TPFP - > 95%
  - Analytical specificity / library of validated MoAbs
  - Clinical sensitivity / TTPF/N - > 95%
  - Analytical sensitivity / LOD - 20 events for reproducible detection
  - Functional sensitivity / LOD - 10 events for reproducible quantification
  - Inter-assay reproducibility / Reproducibility - < 2% / 5%

ICCS/ESCCA Guidelines for the diagnosis and screening of PNH

- There is a role of genetic testing for PNH, using validated genetic panel
- Reduced frequency of PNH clone size remains stable

Screening and Monitoring of GPI-anchor deficiency

- In PNH patients, BMF could develop with BMF or PNH clone
- GPI anchor deficiency with BMF: Trephine biopsy

- PNH Spontaneous remission

- BMF-PNH Spontaneous remission

- BMF-PNH Spontaneous remission

- BMF-PNH Spontaneous remission

- BMF-PNH Spontaneous remission
Analysis of iRBCs (nucleated + reticulocytes) improves the delineation of PNH type III, type II and normal subsets and provides more accurate information concerning the RBC PNH clone size.

**CD235a** - FITC
**CD59** - PE
**CD71** - APC

Sutherland DR, Richards SJ, Ortiz F, Nayyar R, Benko M, Marinov I, Illingworth A. Cytometry B 2019 submitted

**Analysis of iRBCs** improves the delineation of PNH type III, type II and normal subsets and provides more accurate information concerning the RBC PNH clone size.

Marinov et al. 2016

Rare CD157- non PNH cases (< 0.5%) - SNP (Arg145Gln) - Infection (HBV) - Unknown


Analysis of 2 GPI markers per lineage (Ne, Mo) and RBCs:
N2 False positive results reported !!!

Analytical considerations - WBC analysis strategy

5-c (2 laser) FLAER/CD157 based assay

Base: CD157 - non PNH cases (< 0.5%) - Unknown

Analysis of 2 GPI markers per lineage (Ne, Mo) and RBCs:
N2 False positive results reported !!!

Analytical considerations - WBC analysis strategy

6-c FLAER/CD14/CD24 based assay

5-c non-FLAER based assay

Analytical considerations - WBC analysis strategy

6-c FLAER/CD14/CD24 based assay

7-c FLAER/CD14/CD24/CD157 based assay

Analytical considerations - WBC analysis strategy

Post-analytical considerations - reporting

**Reporting**

- GPI deficient clone >1%: PNH clone
- GPI deficient clone 0.1% - 1%: minor PNH clone
- GPI deficient clone <0.1%: GPI phenotype

**Terminology CD11H52-A2**

- GP deficient clone >1%: PNH clone
- GP deficient clone 0.1% - 1%: minor PNH clone
- GP deficient clone <0.1%: GPI phenotype
GPI deficiency testing by HS FCM is useful in young patients to rule out inherited BMFS.

GPI deficiency testing by HS FCM is important to detect and follow up PNH phenotypes in acquired BMFS: 15 - 20% of patients with BMFS could develop subclinical or clinically relevant PNH clones.

GPI deficiency testing by HS FCM is important for predicting response to IST in AA and h-MDS.

GPI deficiency testing by HS FCM is important for predicting progression of AA/h-MDS to MDS/AML.

Mandatory analysis of 2 GPI markers on WBC (Ne, Mo) and RBCs.

Library of validated reagents for various HW configurations.

Report with concern to LOD a LOQ using uniform terminology (CSLI.HS2-421).

Analysis of iRBCs improves the delineation of PNH type III, type II and normal subsets and provides more accurate information concerning the RBC PNH clone size.

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THANK YOU FOR THE ATTENTION