Morphological evaluation of monocytes and monocyte precursors in bone marrow trephine biopsies - need for establishing diagnostic criteria

Goasguen *et al.*[1] in their recent article delineate morphological features that can be used to distinguish four stages of monocytes and their precursors on peripheral blood and bone marrow aspirate films. Evaluation of monocytic lineage cells in hematologic specimens is critical not only on peripheral blood and bone marrow aspirate films, but also in bone marrow trephine biopsies (BMTB). Evaluation of BMTB for a possible monocytic component is particularly sought in the following situations: (i) appreciation of monoblastic features in acute myeloid leukemia (AML) or acute monoblastic and monocytic leukemia (AMoL); (ii) identification and differentiation of chronic myelomonocytic leukemia (CMML) from other myeloproliferative disorders and myelodysplastic syndrome; (iii) subtyping of CMML into CMML-1 and CMML-2.

It is true that the currently evaluated immunophenotypic features are not completely specific for monocytes and monocyte precursors. However, many centers investigate BMTB samples with immunohistochemistry for CD68R and CD163.[2,3] In BMTB of CMML samples, CD68R identifies two populations of monocytic lineage cells. However, CD163 appears to be a more reliable marker for early monocytic differentiation and for lineage identification in "blastic" cells of AMoL.

On BMTB, three stages of monocytic population can be identified (Figure 1):

(i) cells with indented nuclei and absence of nucleoli; on CD68R, these cells have coarser granules;

(ii) cells without indented nuclei but with easily identifiable small nucleoli; on CD68R, these cells have finer granules;

(iii) blastic population of cells, with extremely fine chromatin and sometimes with marked atypia; a greater proportion of these cells express CD163 as compared to CD68R.

The above mentioned monocytes and monocyte precursors appear distinct from phagocytic macrophages and dendritic cells on the CD68R immunostain. As compared to the monocytic cells, the phagocytic macrophages and dendritic cells have more abundant cytoplasm and the staining pattern is one of more uniform and intense staining of the entire cytoplasm. The dendritic processes of the dendritic cells are also highlighted by CD68R. In some of the phagocytic macrophages, the less-stained phagolysosomes are also recognizable on CD68R. It should be emphasized, however, that CD68R expression is also seen in other lineage cells/diseases, like in cells of hairy cell leukemia. Hence, a wider panel of immunostaining and documentation of negative staining with other antibodies, and correlation with other morphological features is essential.

The combined approach of morphology and immunostaining is helpful in identifying the lineage of blasts in cases of AML. In AMoL, not only are >80% of the marrow cells positive for CD68R/CD163, often the proportion of CD163 positive cells exceed the proportion of CD68R positive cells. Furthermore, in suspected cases of CMML, the approach helps in documenting the proportion of monocytic cells in the BMTB sample and in subtyping the cells in two categories - the more mature and the *precursor* subpopulations. The latter show finer granulation with CD68R and have less mature nuclei. This approach helps subtyping CMML into CMML-1 and CMML-2.

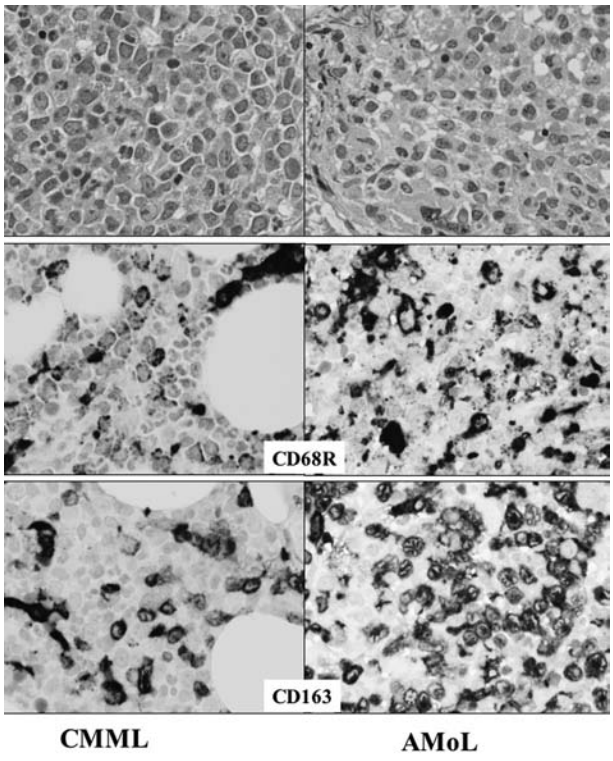

Figure 1. The left panel depicts a case of CMML. A greater proportion of cells are positive for CD68R as compared to CD163. The CD68R positive monocytic cells show granular positivity and the size and distribution of the granules are variable. The more mature monocytic cells have larger granules, while less mature forms have smaller/finer granules. Histiocytic/dendritic cells show intense positivity and some show dendritic processes. The right panel depicts a case of AMoL, where the majority of the cells are atypical and blastic. A greater proportion of the cells are positive for CD163 as compared to CD68R.

Though WHO criteria[4,5] can be applied on BMTB samples with the above-mentioned approach, the criteria need revalidation and subtle modifications for usage on BMTB. Standardization of BMTB processing and quality assurance in BMTB-immunohistochemistry would be of paramount importance.[6] There is a need for establishing morphological and immuno-morphological criteria for diagnosis of hematologic neoplasms on BMTBs. On many occasions, the bone marrow aspirate quality (for various reasons such as marrow fibrosis or other technical reasons) may be suboptimal making a specific diagnosis difficult.

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Chronic hemolytic anemia due to novel α-globin chain variants: critical location of the mutation within the gene sequence for a dominant effect

Approximately 30 hemoglobin (Hb) α-chain variants may entail chronic hemolytic anemia (CHA).[1,2] In some, interaction between heme and globin is hampered, leading to an unstable Hb. In others, the change affects the domain which binds AHSP and associates with the β-chain partner.[3] Variants underlying CHA may also result from in-frame deletion or insertion, leading to a shortened or elongated chain, or from frameshift (FS) deletions and insertions leading to a premature stop codon and a profoundly altered C-terminus.[4]

We herewith report 3 variants associating with CHA in the heterozygous state, a picture in contrast with that of many unstable α chain variants in which only borderline α thalassemia is displayed.[4]

Hb Sens was found in a French Caucasian patient (Table 1). The T>A heterozygous substitution at CD43(α2) changes Phe for Ile at CE1, one of the two invariable residues among all globin chains. This Phe maintains the heme in the proper position for interaction with the globin chain. Missense mutants at this position lead to severe unstable Hbs, with the anemia state aggravated by the consecutive shift of the heme towards the lower oxygen affinity conformation. Comparable variants are Hb Torino (Phe>Ser) and Hb Hirosaki (Phe>Leu) for the α chain, or Hb Hammersmith (Phe>Ser), Hb Sendagi (Phe>Val), and Hb Louisville (Phe>Leu) for the β chain (*see HbVar for details*).[1] It is likely that these mutations do not impair the formation of tetramers but lead to unstable molecules which precipitate into Heinz bodies when submitted to oxidative stress.

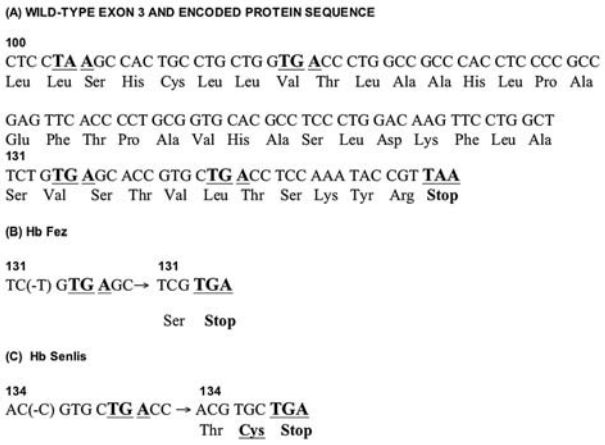

Figure 1. Nucleotide sequence of exon 3 in wild-type and mutated α globin genes, with resulting protein sequences. (A) Wild-type sequences of exon 3 in the α gene showing the potential stops (bold and underlined) that may result from FS. (B) In *Hb Fez*, the 3rd nucleotide of codon 131 is deleted, leading to FS and occurrence of a stop at CD132. (C) In Hb Senlis, deletion of a C within CD134 leads to a stop at CD136 and to a Cys as the outermost residue of the C-terminus.

Hb Fez and *Hb Senlis* (Table 1) are specified by a single nucleotide deletion leading to FS and a premature stop codon. *Hb Fez* was identified in a Moroccan patient with Heinz bodies observed on the blood film, without any visible abnormal Hb. The (-T) deletion, within CDα1-131, leads to a synonymous change at CD132 (TCT>TCG, both encoding Ser), followed by TGA (Stop), and a resulting 131-residue long protein. Hb Senlis was identified in a French Caucasian patient without any apparent abnormal Hb. The (-C) deletion within CDα1-134 leads to FS with two novel residues (Thr and Cys) encoded by codons 134 and 135 (ACG and TGC, respectively), followed by a Stop codon (TGA), and a resulting 135-residue long protein.

Deletion within the 3rd exon has a different outcome whether affecting the α- or β globin-encoding genes. In the α genes, the meaningfulness of the FS is consequent upon its occurrence within the gene sequence (Figure 1). When the deletion involves a nucleotide located between CD100 and 106, a stop is met at position 101 or 107, leading to a protein where helix H is missing, and thus unable to interact with AHSP or the β chain to form α1β1 dimers. Such mutants are likely to be α-thalassemic. A nucleotide deletion occurring between CD107 and 132 (the next potential stop), will more or less significantly alter the structure of helix H, depending how early it occurs within the sequence. As for *Hb Fez* and *Hb Senlis*, with stop codons at positions 132 or 136, respectively, the deleted residues are located by the end of helix H, in a region that does not interact with either AHSP or the β-chain partner.[6] Therefore, an abnormal, unstable, Hb tetramer may likely form and rapidly precipitate within the erythrocyte, accounting for the observed CHA. Furthermore, in *Hb Senlis*, a Cys residue occupies the C-terminus, allowing for possible interchain S-S bonds. When FS occurs more distal within an α2 gene sequence, the next stop is at position 147, leading to Hb Wayne,[7] a relatively stable molecule. In the α1 gene, the nearest potential stop following codon 136 will occur only at position 173, a possibility unreported to this day.

In the β globin gene, deletion of 1 or 2 nucleotides