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Response to Harrison *et al*. 'Clinically relevant differences between BCSH and WHO diagnostic criteria for ET'

Leukemia (2017) **31,** 768–769; doi:10.1038/leu.2016.319

We welcome the critical comments expressed by Harrison *et al.*[1] as they provide an opportunity to address the impact of bone marrow (BM) morphology for the accurate diagnosis of essential thrombocythemia (ET). The readers of *Leukemia* are certainly aware that the first set of the original and updated BCSH diagnostic criteria (A1-A3) allows ET diagnosis without BM morphology examination (see also Table 1—Harrison *et al.*). It is important to highlight that, according to these guidelines, only overt PMF defined by significant BM fibrosis and palpable splenomegaly, blood film abnormalities (circulating progenitors and tear-drop cells) or unexplained anemia is excluded from ET. This explicit restriction to separate only overt PMF from ET disregards early/prefibrotic stages of PMF (prePMF) associated with high platelet counts and summarizes this patient group under the BCSH definition of ET.[2,3] We would like to underscore that prePMF is not recognized and consequently not included in the corresponding updated BCSH-criteria for PMF,[4] contrasting the 2008[5] and recently revised WHO classification.[6]

In our cohort, the diagnostic criteria A1–A3 were met by all BCSH-ET patients. As requested in the BCSH-criteria, PV was excluded by normal hematocrit levels in iron-replete patients. The fact that 16 patients with WHO-PV ended up in the BCSH-ET cohort in our study, only underlines the importance of BM biopsy examination in these patients as recommended in the 2008, and required in the 2016, WHO diagnostic criteria.[5,6] This concerns in particular so-called masked PV,[6] which can be accurately diagnosed through BM biopsy examination in not overtly polycythemic patients. This is further supported by our finding that neither phlebotomy need nor increased red cell mass was observed in these 16 patients at diagnosis, but during follow-up. According to BCSH-criteria, overt PMF is distinguished from ET by the presence of BM fibrosis grade 2/3 or 3/4 accompanied by unexplained anemia, splenomegaly or blood film abnormalities. None of our patients with fibrosis showed any of the mentioned signs and none of the anemic patients were considered as unexplained. However, these features are, along with splenomegaly and elevated LDH levels, commonly found in WHO-prePMF.[2,3]

Dr Harrison and colleagues criticize our inclusion of patients with unknown mutation status in the WHO-ET cohort as a major methodological problem. The 2008 and 2016 WHO-criteria for ET[5,6] do not require the presence of a mutation, but either the exclusion of any reactive cause or a clonal marker. Since reactive thrombocytosis was excluded in all patients, the inclusion of these 91 patients in the WHO-ET cohort in our study was correct.

While Harrison *et al.* note that the survival difference is relatively small and possibly influenced by different age at diagnosis, we would like to underline the differences in the fibrosis-free survival. Median fibrosis-free survival differed significantly between BCSH-ET and WHO-ET (23.6% versus 13.4% after 15 years). This cannot be explained through age at diagnosis alone, but shows that there are substantial differences in the underlying disease in the two groups.

The BCSH labels all of these patients as ET and Harrison *et al.* attribute these differences to different risk profiles and a hitherto little understood molecular heterogeneity. This, however, does not fully explain the significant differences in clinical characteristics and outcome that we and others have found between the two cohorts. The WHO-criteria on the other hand postulate that ET and prePMF are two very different entities.

The authors of the correspondence argue that 'the BCSH criteria were developed because of concerns that the WHO criteria were difficult to apply in a reproducible manner.' Obvious problems and pitfalls regarding this issue have been reviewed in detail[7] and are discussed at length in our paper. Good reliability of morphological features was reported by several groups.[2,3] Contrary to the statement of Harrison *et al.,* the three hematopathologists on the panel were only aware of age and gender, which is needed to assess BM cellularity. Only at a later stage, these diagnoses were compared with the clinical data and only patients in which the clinical diagnosis and histopathological diagnosis coincided were used to recruit the BCSH and WHO cohorts. In the majority of relevant studies, the hematopathologists were either aware of the clinical diagnosis or at least of the suspected diagnosis of a myeloproliferative neoplasm with thrombocytosis. The only exception so far is the blinded study by Madelung *et al.*,[8] including among the 272 biopsy samples and 43 control cases. The final consensus taking into account the clinical data, however, was 83% and not 53% as stated by Harrison *et al.*

The reader of our study has to keep in mind that we did not apply the second set of ET diagnostic criteria (A1+A3 – A5) proposed by the BCSH. Contrasting the first diagnostic scheme, the latter includes BM morphology. (Table 1—Harrison *et al.*). It is evident that in comparison with the WHO-defined morphological

Advance online publication, 29 November 2016

criteria for ET[5,6] the description of the BCSH reveals some striking differences. The failure to recognize the lack of changes of the other hematopoietic cell lineages in comparison with megakaryocytes, including the unspecific term 'spectrum of morphology' is problematic. Moreover, it allows two different grading schemes for the fiber content that may further add to confusion.

It may be argued in hindsight that in practice one would have performed a BM biopsy on exactly those patients that were not classified as ET by the WHO-criteria. However, this raises the question: what would be the consequence of this biopsy in patients without an overt fibrosis? Again, this patient group has to be classified according to the BCSH-criteria as ET as the BCSH-criteria do not recognize prePMF as an entity, separate from that of ET.[1,4]

Finally, we fully agree with Dr Harrison *et al.* that thrombocythemic MPN are a heterogenic group of patients with different clinical and biological characteristics. However, without an exact differentiation, including BM biopsy examination, we never will be able to understand their potential differences in molecular genetic characteristics.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Response to 'Overexpression of *ABCB1* as prediction marker for CML: How close we are to translation into clinics?'

Leukemia (2017) **31,** 769–770; doi:10.1038/leu.2016.335

The recently published letter from Némethová and Rázga[1] makes the valid suggestion that the predominant cell type in patient samples may have bearing on ABCB1 expression. We have subsequently investigated the constituent cell types from the 155 patients included in our study.[2] Lymphocyte, monocyte, basophil, eosinophil and neutrophil cell population percentages were determined in all patients at diagnosis. In order to ascertain whether the predominant cell type prior to imatinib therapy may influence long-term therapy outcome, proportions of each of these cell types in patients with low-fold-rise ($<$2.2-fold) *ABCB1* mRNA at day 22 vs day 1 were compared with percentages from patients with high *ABCB1* mRNA fold rise ($\geqslant$2.2-fold). Indeed, patients with a low fold rise in *ABCB1* mRNA who were more likely to achieve good therapy outcomes (Early Molecular Response, Major Molecular Response, MR4.5) had a significantly higher percentage of lymphocytes compared with patients who exhibited a high fold rise in *ABCB1* mRNA and subsequently failed to achieve therapy benchmarks (Figure 1a, $P<0.001$). Conversely, there was no difference in percentages of monocytes, basophils, eosinophils or neutrophils between the two patient groups (Figure 1b-e, $P>0.05$).

In our original study we postulated that clonal selection of ABCB1-expressing granulocytes contributed to the increased ABCB1 levels observed in patients from the high *ABCB1*-fold change group. Unfortunately, cell population data are not available for day 22, so we are unable to ascertain changes in the percentage of granulocytes (basophils, eosinophils, neutrophils) in response to imatinib therapy. We observed that, at day 1, the majority of patients tended to express two populations of granulocytes regardless of the ABCB1-fold rise group to which they belonged: ABCB1-positive and ABCB1-negative. Conversely, at day 22, patients with a high fold rise in *ABCB1* mRNA were more likely to harbour ABCB1-positive granulocytes, while in patients with a low fold rise in *ABCB1* mRNA the ABCB1-negative granulocyte population had increased. Thus, we hypothesised that the change in ABCB1 occurred via clonal selection of ABCB1-

Advance online publication, 2 December 2016