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Regulation of serum hepcidin levels in sickle cell disease

The peptide hormone hepcidin exerts its function by binding to the transmembrane cellular iron exporter ferroportin and inducing its internalization and degradation, resulting in decreased intestinal iron uptake and iron retention in the reticulo-endothelial (RE) macrophages. Inflammatory cytokines and iron loading increase hepcidin production, while increased bone marrow activity, and anemia suppress hepcidin synthesis. However, most of the evidence of these regulatory processes is obtained by molecular in vitro work and mice models, and much is still unknown about how these different stimuli interact in man.

Sickle cell disease (SCD) patients are characterized by chronic hemolytic anemia, increased erythropoiesis and a chronic inflammatory state with endothelial activation and enhanced red cell and leukocyte adhesion. Sickle cell patients have iron overload due to chronic blood transfusions in the treatment or prevention of the severe sickle cell-related complications such as stroke. SCD has been associated with low urinary hepcidin levels in children. However, serum hepcidin 25-amino acid isoform (hepcidin-25) levels, which are directly responsible for the biological effect, have not been documented and factors that contribute to hepcidin regulation in this disease have not been assessed.
β patients (HbSS, HbS-HbS) were assessed to delineate the reg-

-mers, this ratio might not be suitable in the evaluation of the adequacy of hepcidin in response to hepatocyte iron loading.

Results confirm that erythropoiesis down-regulates hepcidin-25, i.e., when only sTfR is increased, serum hepcidin-25 levels are in the lower normal range or even not detectable (<LLOD-3.6 nM; patients 2, 4, 5, 13-16). In cases where next to a substantially increased sTfR inflammation and/or high iron stores are also present, serum hepcidin-25 levels are in the normal range (1.2 - 9.5 nM; patients 1, 3, 6, 8-12) confirming the induction of hepcidin by inflammation and elevated iron stores in sickle cell patients. Interestingly, in patient 7 the low hepcidin-25 level due to increased erythropoiesis (highly elevated sTfR) is not compensated by low grade inflammation (CRP of 10 mg/L) and a slightly elevated iron store (ferritin of 210 µg/L), resulting in undetectable serum hepcidin-25 levels.

While this is a small study, the results only describe the qualitative contribution of the various parameters to hepcidin-25 levels. Nevertheless, Spearman’s correlation analysis showed that serum hepcidin-25 levels were significantly correlated with urine hepcidin-25, log ferritin, Body Mass Index (BMI)56 (Figure 1A-C) and age, but not with CRP, sTfR, TS (Figure 1D-F) and hemoglobin.

In conclusion, this proof of principle study in a heterogeneous group of SCD patients indicates that: (i) previous results obtained in vitro and mice studies of hepcidin-25 suppression by increased erythropoietic activity that is counterbalanced by iron stores and (low grade) inflammation are also valid in man; (ii) larger studies are needed to determine the quantitative contribution of various factors to hepcidin-25 regulation in this disease.

The insights gained in this study could be clinically beneficial in the identification and treatment of patients most at risk of iron mediated tissue damage.

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Letters to the Editor

Predictive value of β2-microglobulin (β2-m) levels in chronic lymphocytic leukemia since Binet A stages

We read with interest the study by Rossi and co-workers, reporting CD49d expression as risk factor of treatment free survival (TFS) in Binet A CLL patients.1 In this paper, a close association between CD49d and CD38, LDH and β2-m is described. We would like to add further information about the prognostic power of β2-m. It is generally believed that β2-m is released constitutively by CLL cells and that its level approximately correlates with tumor mass.2 Based on these premises the predictive value of β2-m serum concentration could vary in the course of the disease and be relatively low in the early disease stages, when tumor mass is low, irrespective of the subsequent clinical outcome. Therefore, β2-m determination could exhibit a lower predictive power particularly at the early disease stages compared to the newer biological markers, such as IgVH gene status, ZAP-70 and CD38, which represent intrinsic cell features that can be determined since the neoplastic cell burden is low, irrespective of the subsequent clinical outcome. Thus, β2-m determination could exhibit a lower predictive power particularly at the early disease stages compared to the newer biological markers, such as IgVH gene status, ZAP-70 and CD38, which represent intrinsic cell features that can be determined since the neoplastic cell burden is low, irrespective of the subsequent clinical outcome. Thus, β2-m determination could exhibit a lower predictive power particularly at the early disease stages compared to the newer biological markers, such as IgVH gene status, ZAP-70 and CD38, which represent intrinsic cell features that can be determined since the neoplastic cell burden is low, irrespective of the subsequent clinical outcome.

To explore this issue, we have measured β2-m value in 222 Binet stage A patients at diagnosis. IgVH gene status and CD38 expression were also determined in all cases studied. Unlike β2-m, which was measured at diagnosis, these markers were determined in the course of the disease when marker determinations became available. This approach, although irrelevant for the IgVH gene status, may introduce some, albeit minor, biases for CD38 for the reasons alluded to above. The median β2-m value was 2 mg/dL (range 0.4-19). ROC analysis determined that the cut-off value capable of discriminating between patients whose disease progressed and required treatment from those with stable disease was 2.4 mg/dL (AUC=0.67, p=0.005). Accordingly 149/222 patients (67%) were β2-m>30% and 73/222 (33%) as β2-m≤30%. Overall, the results did not substantially change when arbitrary cut offs used by other authors1 were employed.

The patients’ features are summarized in Table 1. β2-m levels overlap with CD38 expression in 128/219 cases (63%). [β2-m<30%/CD38≤30% cases: 23/55 (41.8%), β2-m≥30%/CD38>30% cases: 115/164 (70.1%)], while β2-m levels overlap with IgVH status in 125/195 cases (64.1%). [β2-m<30%/IgVHmutated cases: 29/62 (46.8%), β2-m≥30%/IgVHmutated: 96/133 (72.2%)]. Finally, the concordance between CD38 expression and IgVH mutational status was 77.6% (149/192 cases) (IgVHmutated/CD38≤30% cases: 35/52 (67.3%), IgVHmutated/CD38>30% cases: 114/140 (81.4%).

After a median follow-up of 3.5 years, 55 of 222 Binet stage A (25%) required treatment. β2-m>30% cases showed a significantly longer TFS than β2-m≤30% cases; in particular the projected median TFS was 5.3 years for β2-m≤30% versus not reached for β2-m>30% (Figure 1A). TFS represented a reliable measure of disease progression since all centers agreed to follow NCI guidelines for treatment start.

In order to ascertain whether β2-m identifies a patient subset with good prognostic markers, we calculated TFS of both CD38≤30% and IgVHmutated CLL cases grouped according to the β2-m expression. β2-m≤30% CD38≤30% cases exhibited a TFS which was significantly lower than that of β2-m<30% CD38≤30% cases (3.5-years TFS probability: β2-m≥30% vs. β2-m≤30% 91% vs. 83%; p=0.05). However, these differences were not seen in the IgVHmutated cases (3.5-years TFS probability: β2-m<30% vs. β2-m≤30% 89% vs. 84%; p=ns).

At Cox univariate analysis, β2-m<30% (HR:2.3, p=0.003), CD38≤30% (HR:3.9, p<0.0001) and IgVHmutated (HR:3.2, p<0.0001) showed a statistically significant impact on TFS. At Cox multivariate analysis, all the three markers maintained an independent prognostic impact (β2-m<30%, HR:1.8, p=0.047; CD38≤30%, HR:2.0, p=0.03; IgVHmutated, HR:2.7, p=0.022). When a scoring system in which one point was assigned to each unfavorable prognostic marker was utilized, the risk of an early treatment was highest (Figure 1B) in patients presenting all the three adverse prognostic markers. Cases with two, one or none of the unfavorable prognostic factors showed lower risk for an early treatment (Figure 1C).

Collectively, this study shows that β2-m levels represent valuable predictors in early CLL stages, when the neoplastic cell burden is low. This finding raises a number of questions regarding the mechanisms governing the β2-m levels. This molecule is constantly shedded.

Table 1. Comparisons of clinical and laboratory features among chronic lymphocytic leukemia patients devised according to β2-m expression.

<table>
<thead>
<tr>
<th>N of patients</th>
<th>All patients</th>
<th>β2-m &lt;2.4 mg/d</th>
<th>β2-m ≥2.4 mg/d</th>
<th>p</th>
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</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>&lt;65</td>
<td>124 (56)</td>
<td>94 (63)</td>
<td>30 (41)</td>
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<td></td>
<td>&gt;65</td>
<td>98 (44)</td>
<td>55 (37)</td>
<td>43 (59)</td>
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<tr>
<td>Gender</td>
<td>Female</td>
<td>82 (37)</td>
<td>60 (40)</td>
<td>22 (30)</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>140 (63)</td>
<td>99 (60)</td>
<td>51 (70)</td>
</tr>
<tr>
<td>IgVH mutational status (n=195)</td>
<td>Mutated</td>
<td>133 (68)</td>
<td>96 (74)</td>
<td>37 (56)</td>
</tr>
<tr>
<td></td>
<td>Germine</td>
<td>62 (32)</td>
<td>33 (26)</td>
<td>29 (44)</td>
</tr>
<tr>
<td>CD38 expression (n=219)</td>
<td>&lt;30%</td>
<td>164 (75)</td>
<td>115 (78)</td>
<td>42 (58)</td>
</tr>
<tr>
<td></td>
<td>≥30%</td>
<td>55 (25)</td>
<td>32 (22)</td>
<td>30 (42)</td>
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<td>Therapy</td>
<td>no</td>
<td>167 (75)</td>
<td>123 (83)</td>
<td>44 (60)</td>
</tr>
<tr>
<td></td>
<td>yes</td>
<td>55 (25)</td>
<td>26 (17)</td>
<td>29 (40)</td>
</tr>
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