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LETTER TO THE EDITOR

Elevated levels of serum CD44 and E-cadherin predict an unfavourable outcome in myelodysplastic syndromes

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Myelodysplastic syndromes (MDS) represent a heterogeneous group of clonal haematopoietic stem cell disorders with variable clinical course depending on ineffective haematopoiesis or transition to acute myeloid leukaemia (AML).[1] Prognosis of MDS is closely correlated with bone marrow blast cell count, cytogenetic abnormalities and the degree of cytopenia, expressed in the International Prognosis Scoring System (IPSS). However, it is still difficult to predict survival in individual patients and the definition of additional parameters remains relevant for decisions in MDS-directed therapies.

The cell–cell adhesion molecule E-cadherin (120 kDa) and cell–matrix adhesion molecule CD44 (90 kDa) are involved in the regulation of haematopoiesis and are expressed on diverse types of mature blood cells and CD34+ stem cells.[2,3] Their aberrant expression plays a pivotal role in various solid and haematological malignancies like multiple myeloma, AML and lymphoma.[4–7] In malignant cells, these membrane-anchored proteins undergo activated proteolysis of the extracellular domain resulting in elevated serum levels of the soluble and bioactive polypeptides serum CD44 (sCD44) (25 kDa) or serum E-cadherin (sE-cad) (80–84 kDa).[4] The multifunctional properties of both adhesion molecules with expression on diverse types of blood cells suggest a critical role in the pathogenesis of MDS. Takubo *et al*.[8] described elevated serum levels of E-cadherin in a small MDS cohort ($n=13$) without analysis of its clinical significance. Similarly, there is also only one study reporting slightly increased CD44 serum levels in MDS ($n=43$),[9] without subgroup analysis and no investigation of its impact on survival. In this report, the prognostic significance of serum levels of sCD44 standard (sCD44 s), the variant isoform CD44v6 (sCD44v6) and E-cadherin (sE-cad) was analysed in a cohort of 66 MDS patients. The study was approved by the local ethics committee and comprised clinical data and serum samples from consecutive, newly diagnosed and treatment-naive MDS patients who had given written informed consent before any study procedure. Patients with severely impaired liver or kidney function or elevated C-reactive protein were excluded from the study. MDS stage was classified according to the criteria of the French-American-British (FAB) group.[1] Median age of patients was 71 years (range 19–93 years), female to male ratio 1:1.44. Fifteen patients presented with refractory anaemia (RA), five patients with RA with ringed sideroblasts (RARS), 21 patients with RA with excess of blasts (RAEB), 11 patients with RAEB in transition (RAEB-t), nine patients with chronic myelomonocytic leukaemia (CMML) and five patients with overt secondary acute leukaemia. The control group consisted of 19 age-matched persons without any evidence of inflammatory or neoplastic disease. Quantification of sCD44 s, sCD44v6 and sE-cad in serum samples was determined by enzyme-linked immunosorbent assay following the manufacturer's instruction (Bender MedSystems, Vienna, Austria; R&D Systems, Minneapolis, MS, USA). Statistical analysis was performed using SPSS 12.0 software (SPSS Inc., Chicago, IL, USA). The two-sided Mann–Whitney *U*-test was used to compare means between different FAB groups. Correlation coefficients were calculated with Spearman's rank correlation. Survivals were plotted by the Kaplan–Meier (KM) method and compared by the log-rank test. The observation period lasted at least 1 year. Cutoff levels for sCD44 s and sE-cad were defined as the mean level of healthy persons plus 2 s.d. Multivariate survival analysis was performed using the Cox proportional hazard model, including sCD44 s, sE-cad, age, sex, lactate dehydrogenase (LDH) and IPSS. Based on the hazard ratio, the mortality risk for an individual patient depending on levels of sCD44 s or sE-cad was calculated by hazard ratio (95% confidence interval) (increase of serum value in ng/ml).

Levels of serum CD44 s and E-cadherin were significantly elevated in patients with MDS as compared to healthy donors (Figure 1), whereas sCD44v6 showed no significant difference.

sCD44 s was characterized by a differential expression in various FAB subgroups, revealing the highest levels in CMML and in secondary AML (Figure 1), thus extending data from previous reports.[9] Median level of sCD44 s in controls was 487 ng/ml (range 325–890 ng/ml), in RA 736 ng/ml (range 417–1210 ng/ml), in RARS 598 ng/ml (range 493–911 ng/ml), in RAEB 638 ng/ml (range 301–1077 ng/ml), in RAEB-t 774 ng/ml (range 417–1138 ng/ml), in CMML 1470 ng/ml (range 599–7961 ng/ml) and in secondary AML 960 ng/ml (range 459–1757 ng/ml).

sE-cad was elevated in patients with RA, RAEB and CMML, but not in those with RAEB-t or secondary AML (Figure 1). Median level of sE-cad in controls was 83 ng/ml (range 57–188 ng/ml), in RA 115 ng/ml (range 67–286 ng/ml), in RAEB 111 ng/ml (range 55–221 ng/ml), in CMML 136 ng/ml (range 87–230 ng/ml), in RARS 104 ng/ml (range 79–131 ng/ml), in RAEB-t 87 ng/ml (range 60–146 ng/ml) and in secondary AML 95 ng/ml (range 63–128 ng/ml). The lack of significant elevation of sE-cad levels in MDS with increased bone marrow blasts (RAEB-t or sAML) could point to silencing of E-cadherin expression in leukaemogenesis.[10] In acute myelogeneous leukaemia, hypermethylation of the E-cadherin gene and consequent loss of E-cadherin RNA and protein has been demonstrated.

We report for the first time, that both circulating adhesion molecules are highest present in CMML. The clinical relevance of strong activation of soluble adhesion molecules might be expressed in the frequent development of extramedullary infiltrations in patients with CMML, involving spleen, skin, lung and brain. Recently, ligation of CD44 with specific anti-CD44 monoclonal antibodies emerged as an efficient way to inhibit the proliferation of AML cells. Thus, CMML could also be a candidate for CD44-targeting therapeutic concepts.

Levels of sCD44 s and sE-cad were not associated with the number of bone marrow blasts, cytogenetics, IPSS, platelet counts, haemoglobin or LDH level, age or sex, but were correlated with elevated leucocyte count ($r=0.454$, $P<0.001$ and $r=0.273$, $P=0.03$, respectively), especially with the

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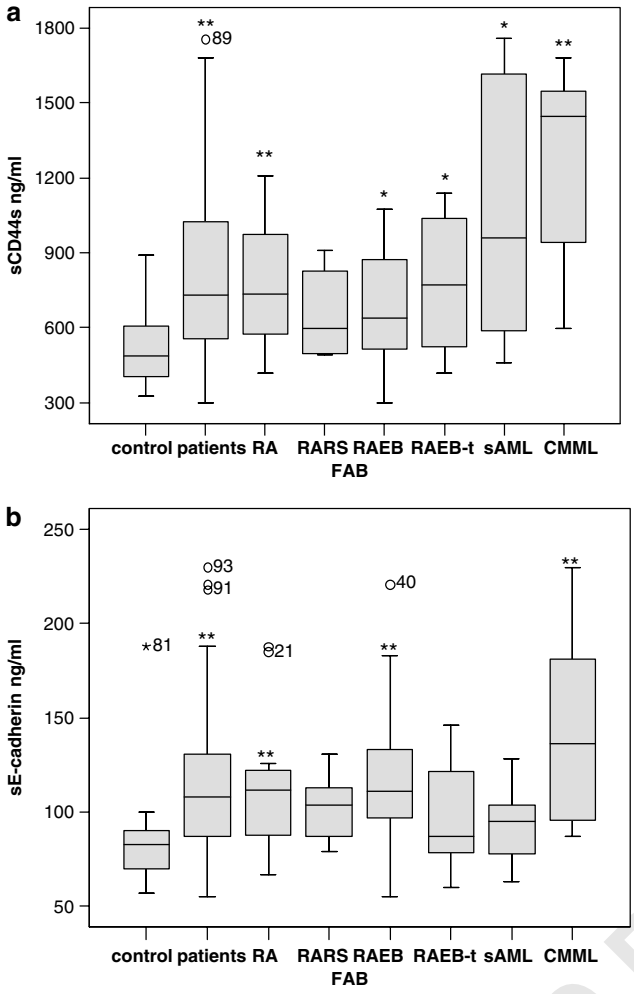

Figure 1 Differential expression of sCD44 s (**a**) and sE-cad (**b**) in 66 MDS patients (RA: 15, RARS: 5, RAEB: 21, RAEB-t: 11, CMML: 9, secondary AML: 5), and in 19 controls. Box plots represent 25th and 75th percentiles as box. The bar indicates the median value. Median levels of controls and patients were compared with Mann–Whitney *U*-test, $^{**}=P<0.01$ and $^{*}=P<0.05$. For display purposes, two CMML patients with extraordinary high sCD44 s values are not depicted in the sCD44 s box plot (Nos. 6 and 8 with 7961 and 2773 ng/ml, respectively).

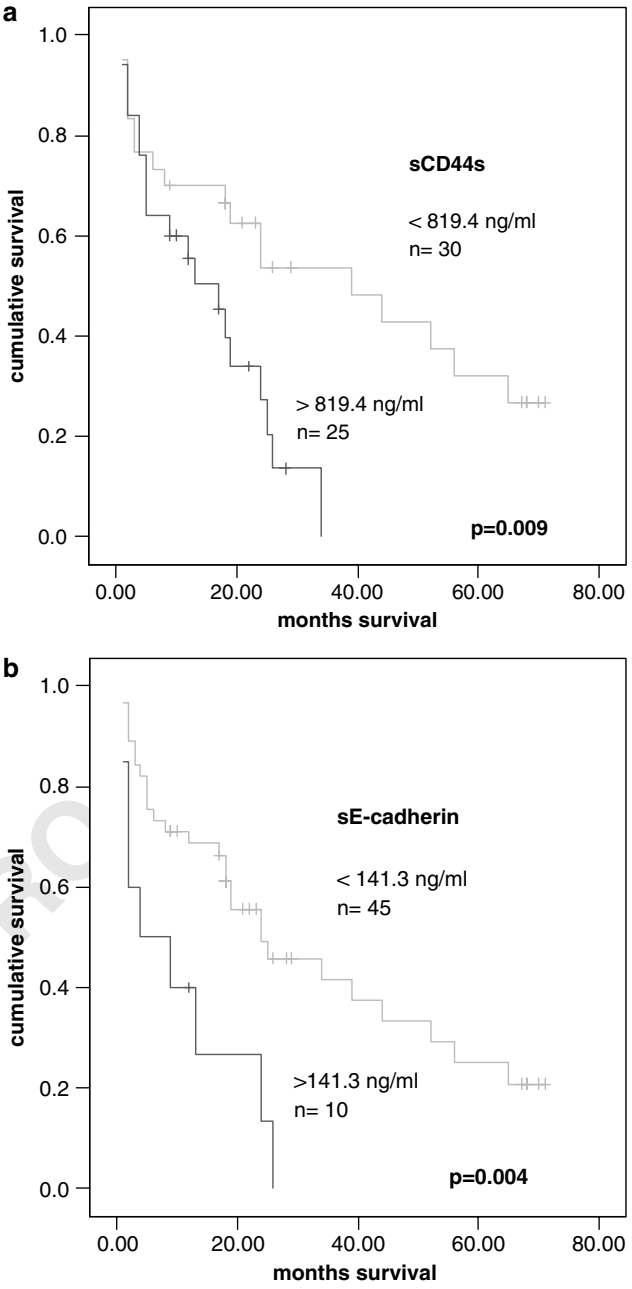

Figure 2 Elevated serum levels of sCD44 s (**a**) and sE-cad (**b**) are associated with shorter survival in MDS patients, as analysed by KM procedure. Cutoff levels were defined on the basis of healthy controls plus 2 s.d.

monocyte count ($r=0.583$, $P=0.05$ and $r=0.77$, $P=0.016$, respectively). This finding was most expressed in the CMML group, in the hyperproliferative as well as in the hypoplastic subtype.

To analyse the prognostic relevance of both markers, KM plots were performed, excluding patients with secondary AML or patients who underwent stem cell transplantation.

Increased sCD44 s or sE-cad levels (cutoff level 819.4 or 141.3 ng/ml, respectively) correlated with shortened survival (17 versus 39 months survival, $P=0.009$ or 4 versus 24 months, $P=0.004$, respectively; Figure 2a and b). KM analysis also confirmed the prognostic value of IPSS. Patients with low/intermediate-1 risk (IPSS 0-1) revealed a median survival of 25 months, whereas patients suffering from intermediate-2/high risk MDS (IPSS 1.5–3.5) were characterized by a median of only 5 months ($P=0.008$). The sample size of our cohorts limits further subgroup analysis. However, there is a trend that especially patients with RA, RAEB or CMML otherwise characterized by a favourable karyotype and low IPSS score (0–1) displayed a poorer prognosis associated with high sCD44 s or sE-cad levels. In a Cox proportional hazard model, the IPSS score was confirmed as the most relevant prognostic factor. In addition, sCD44 s and sE-cad were identified as independent predictors (Table 1). Accordingly, based on *hazard ratio* an increase in sCD44 s from 500 to 1500 ng/ml would result in an up to 2.7-fold risk of mortality. Similarly, elevation of sE-cad from 80 to 140 ng/ml would reflect an up to 5.2-fold risk of death.

In conclusion, this is the first study providing evidence for a prognostic value of the adhesion molecules CD44 and E-cadherin in MDS. Distinct FAB subtypes revealed differential

Table 1 Risk of death in MDS patients by increase of serum levels of CD44 s or E-cadherin per ng/ml, as calculated using a Cox regression model and adjusted for IPSS, LDH, age and sex

Variable	*Hazard ratio (95% CI)*	P-*value*
sCD44 s (per ng/ml)	1.000 (1.000–1.001)	0.013
sE-cadherin (per ng/ml)	1.017 (1.005–1.028)	0.004
age (per year)	1.010 (0.985–1.036)	0.442
IPSS (per point)	2.323 (1.522–3.546)	0.000
LDH (per U/l)	1.003 (0.997–1.003)	0.959
Sex	0.520 (0.236–1.145)	0.104

Abbreviations: CI, confidence interval; IPSS, International Prognosis Scoring System; LDH, lactate dehydrogenase; MDS, myelodysplastic syndromes.
Only IPSS, CD44 and E-cadherin serum levels emerged as independent prognostic factors. Accordingly, an increase of sCD44 s from 500 to 1500 ng/ml results in an up to 2.7-fold risk of mortality. Similarly, elevation of sE-cadherin from 80 to 140 ng/ml translates into an up to 5.2-fold risk of death, calculated by hazard ratio (increase in ng/ml).

sCD44 s and sE-cad expression, with CMML exerting the highest levels of both adhesion molecules. Elevated levels of sCD44 s or sE-cad (cutoff level >819.4 ng/ml or >141.3 ng/ml, respectively) were correlated with shorter survival and reflected prognostic significance independent from IPSS, which should be further tested in risk stratification models and prospective studies.

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