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Increased granulocyte membrane neprilysin (CD10) expression is associated with better prognosis in heart failure

The inhibition of the ubiquitous transmembrane enzyme neprilysin (NEP) by angiotensin receptor-neprilysin inhibitor (ARNI) therapy represents a novel mechanisms in the combat against heart failure with reduced ejection fraction (HFrEF).[1] NEP exerts pleiotropic effects on numerous regulatory peptides, rendering the exact mechanism of action still a subject of debate. The soluble form of the enzyme (sNEP), which can be detected in plasma, is similarly discussed controversially as a potential biomarker in HFrEF.[2] NEP is not only present in solid tissues but is identical to CD10, expressed on the

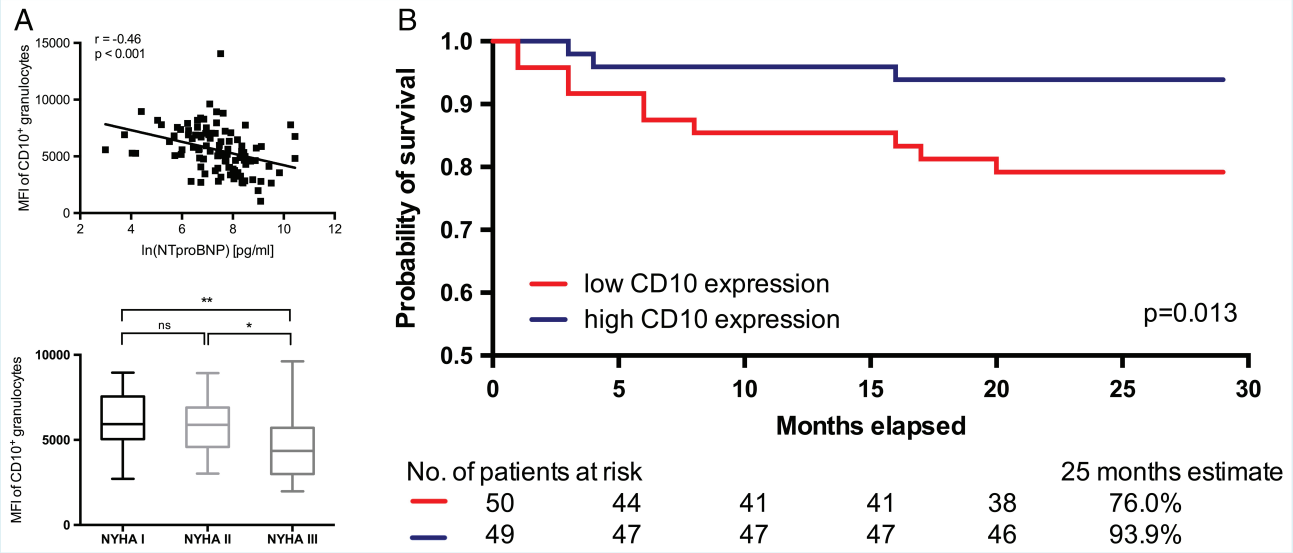

Figure 1 Association of granulocyte neprilysin (CD10) expression with heart failure severity and prognosis. (*A*) Scatter plot with linear regression analysis and the Spearman rho correlation coefficient for mean fluorescence intensities (MFI) of $CD10^+$ granulocytes with N-terminal pro B-type natriuretic peptide (NT-proBNP) as well as group comparisons between New York Heart Association (NYHA) class are shown. (*B*) Kaplan–Meier analysis for heart failure with reduced ejection fraction patients with low and high granulocyte neprilysin (CD10) expression with the median MFI as the cut-off value. Comparison was calculated by the log-rank test. * $P < 0.05$; ** $P < 0.01$.

surface of leucocytes under physiological conditions.[3] Alterations of NEP expression have been reported in sepsis, whereas CD10 was suggested to be indicative for neutrophil functional capacity.[4] Nevertheless, up to now no data on membrane granulocyte NEP expression in HFrEF exist.

A total of 99 consecutive patients with stable chronic HFrEF and optimal medical therapy, including angiotensin-converting enzyme inhibitors or angiotensin II receptor blockers (ACE-I/ARB), but not ARNI, have been enrolled prospectively. CD10 expression on peripheral leucocyte subsets was measured by flow cytometry in 100 μL EDTA whole blood using a combination of six antibodies with fluorescence minus one sample as control [CD3(#555339), CD19(#555413), CD56(#335826), CD16(#561306), CD14(#562692), +/− CD10(#332777); BD Biosciences, San Jose, CA, USA]. Additionally, soluble NEP concentrations were analysed using a specific enzyme immunosorbent assay (SEB785Hu, USCN, China). Demographic and routine laboratory data including New York Heart Association (NYHA) class and N-terminal pro B-type natriuretic peptide (NT-proBNP) were recorded and all-cause mortality was assessed as the primary endpoint. The protocol was approved by the local ethics committee. Continuous data are presented as median and interquartile range (IQR), categorical data as counts and percentages. Medians between groups were compared using the Kruskal–Wallis test and the Mann–Whitney U test. The Spearman rho correlation coefficient was calculated for the correlation between NEP expression and NT-proBNP or sNEP. Univariate and adjusted Cox proportional hazard regression analysis was used to evaluate the effect of NEP expression on all-cause mortality.

Median age was 65 years (IQR 55–73), 75% of patients were male, and beta-blocker, ACE-I/ARB and mineralocorticoid receptor antagonist therapy was established for 96%, 95% and 77% of patients, respectively. Median NT-proBNP levels were 1700 pg/mL (IQR 794–4009). NEP was markedly expressed on granulocytes with 94.8% (IQR 90.5–97.4) of $CD10^+$ cells and measurable on B-cells and monocytes with 8.5% (IQR 5.3–13.5) and 0.8% (IQR 0.4–1.5) of $CD10^+$ cells of the respective leucocyte subtype. NEP expression on T-cells was not detectable. The mean fluorescence intensity (MFI) of $CD10^+$ cells was 5461 (IQR 4028–6904) for granulocytes, 640 (IQR 535–740) for B-cells, and 1589 (IQR 1395–1975) for monocytes. Granulocyte NEP expression, but not NEP expression on B-cells or monocytes, correlated inversely with heart failure severity reflected by NT-proBNP levels ($r = -0.46$, $P < 0.001$) and NYHA class ($P = 0.013$) (*Figure 1A*). sNEP concentrations correlated weakly with NEP expression on granulocytes ($r = 0.22$, $P = 0.030$) as well as the MFI of $CD10^+$ granulocytes ($r = 0.31$, $P = 0.003$). Fifteen (15%) out of 99 patients died during a median follow-up of 24 (IQR 23–28) months. Increased NEP expression on granulocytes was indicative for better overall survival in the univariate model [crude hazard ratio (HR) per 1 IQR increase of MFI 0.40, 95% confidence interval (CI) 0.17–0.90; $P = 0.027$] and after adjustment for age and kidney function (adjusted HR per 1 IQR increase of MFI 0.41, 95% CI 0.18–0.94; $P = 0.035$). Kaplan–Meier analysis illustrates the impact of granulocyte NEP expression on outcome graphically (*Figure 1B*).

In conclusion, albeit beneficial effects of NEP inhibition by ARNI therapy, NEP expression on granulocytes is inversely correlated with heart failure severity and mortality. The results support the inverse relationship between B-type natriuretic peptide and plasma NEP activity reported for a mixed population of heart failure patients.[5] The positive correlation of granulocyte NEP expression and sNEP indicates a possible contribution of shed membrane NEP molecules to plasma NEP levels as a surrogate marker. The utility of granulocyte NEP expression or sNEP as biomarkers in HFrEF has to be further evaluated.

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