

Low serum gamma-glutamyltranspeptidase in patients with chronic idiopathic neutropenia is not implicated in the pathophysiology of the disease

Increased apoptosis of bone marrow granulocyte progenitor cells has been implicated in the pathophysiology of chronic idiopathic neutropenia (CIN) but the molecular abnormality underlying the cellular defect is unknown. In this report we study serum gamma-glutamyltranspeptidase (GGT) levels and GGT mRNA expression in peripheral blood cells of patients with CIN. We found that the low serum GGT in the patients is associated with normal GGT mRNA expression and we concluded that the low levels of the enzyme are probably due to the decreased number of circulating neutrophils and memory T-lymphocytes in CIN.

Haematologica 2007; 88(11):e157-e158

Chronic idiopathic neutropenia (CIN) is an acquired granulocytic disorder characterized by a prolonged "unexplained" reduction of the number of circulating neutrophils below the lower limit of the normal range for a given ethnic population.^{1,2} The disorder mainly affects middle-aged women and is typically characterized by a low incidence of infections, normal bone marrow (BM) cell karyotype, negative tests for anti-neutrophil antibodies, and a HLA class II genetic predisposition.^{2,3} We have recently shown that CIN patients display impaired granulopoiesis due to accelerated apoptosis of BM CD34⁺/CD33⁺ granulocyte progenitor cells. Although a consistent inhibitory effect of the patients' BM microenvironment has been demonstrated, it remains unclear whether an underlying molecular defect renders BM granulocyte progenitors more vulnerable to apoptotic stimuli.⁴ Data from our Neutropenia Unit are suggestive of low serum gamma-glutamyltranspeptidase (GGT) levels in CIN patients. GGT is an ectopeptidase present in a variety of cell types including hematopoietic cells.⁵ By catalyzing the hydrolysis and transfer of g-glutamyl moieties from extracellular g-glutamyl peptides to intracellular amino acids or peptide acceptors, GGT has been considered to play a pivotal role in regulating glutathione homeostasis and intracellular redox state, therefore protecting cells from apoptosis.^{5,6} Three types of GGT mRNA, derived from at least five loci on chromosome 22q11 proximal to the breakpoint cluster region (bcr) gene, have been described.^{7,8} Type I mRNA encodes for the active GGT ubiquitously expressed in human tissues, type II mRNA is associated with multiple point mutations and actually no protein translation, while type III mRNA is truncated and its translation product does not exhibit any GGT-related activity. Interestingly, truncated type III mRNA is identified in blast cells of about 50% of cases of acute lymphoblastic leukemia.⁸ To examine the cause and the possible involvement of low serum GGT in the pathophysiology of CIN, we studied 102 CIN patients, 17 men and 85 women aged 15 to 78 years (median 52 years) with a mean neutrophil count 1445±300 per mL of blood (median 1500, range 393 to

1776 neutrophils per mL of blood) and 72 age- and sex-matched healthy controls with a mean neutrophil count 3978±996 per mL blood (median 3874, range 2647 to 6410 neutrophils per mL of blood). Informed consent according to the Helsinki declaration was obtained from all subjects studied. In particular, we evaluated serum GGT levels by means of a routine enzymatic method and we also studied GGT mRNA expression in peripheral blood cell extracts from 18 selected CIN patients with very low GGT levels by means of reverse transcriptase polymerase chain reaction (RT-PCR). Previously described primer sets Amp1 (5'-AAT-GGA-CGA-CTT-CAG-CTC-TCC-3') and Amp2 (5'-AGC-CGA-ACC-AGA-GGT-TGT-AGA-3) were utilized along with Amp1 and Amp5 (5'-AAA-GGG-GTG-ACA-CAT-ATC-AG-3') to amplify type I and type III GGT mRNA, respectively.^{7,8} Conditions for 35 cycles of PCR amplification following initial denaturation were 94°C for 1 min, 55°C for 1 min, and 72°C for 2 min. We found that serum GGT levels were significantly lower in CIN patients (mean 12.6±3.9 IU/L, median 12.0 IU/L, range 7.0 to 29.0 IU/L) compared in the controls (mean 19.7±8.4 IU/L, median 18.0 IU/L, range 9.0 to 47.0 IU/L) ($p < 0.0001$). Individual GGT values strongly correlated with both the number of circulating neutrophils ($r = 0.542$, $p < 0.0001$) and the num-

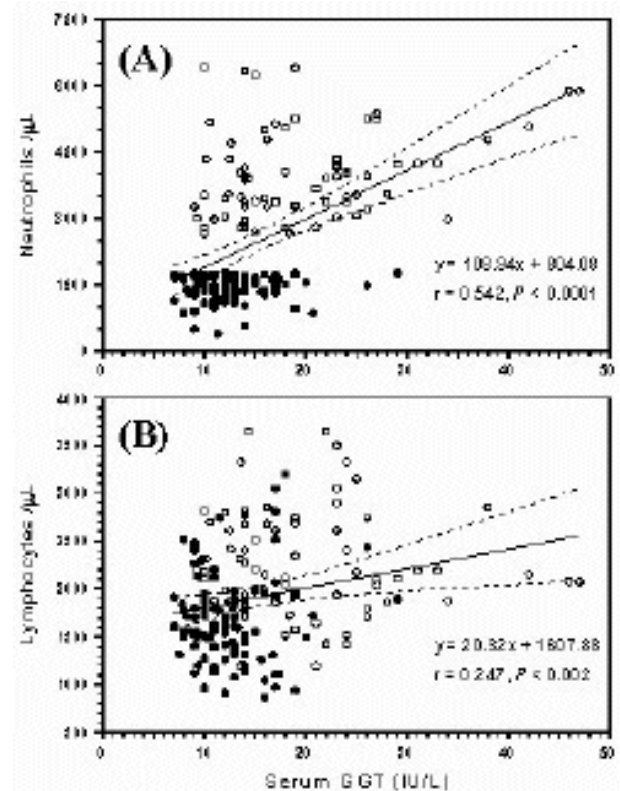


Figure 1. Strong correlation between the levels of serum GGT and the number of circulating neutrophils (A) and lymphocytes (B) in the entire group of subjects studied (Pearson's test). The regression line is shown as a solid line and the 95% confidence limits as dotted lines.

ber of lymphocytes ($r=0.247$, $p<0.002$) (Figure 1). CIN patients expressed normal GGT type I mRNA in peripheral blood cells. Truncated (type III) mRNA was not detected in either the patients or the controls. These findings suggest that abnormalities in GGT seem unlikely to be implicated in the pathophysiology of CIN. The low serum GGT in the patients is probably the result rather than the cause of neutropenia reflecting the low number of circulating neutrophils in CIN. The strong correlation between the values of serum GGT and the number of circulating neutrophils as well as the lack of type III mRNA expression that might have an impact on normal enzyme production and function, corroborate this assumption. Furthermore, we postulate that the correlation between serum GGT and peripheral blood lymphocytes probably reflects the low number of memory CD45RO⁺ T-cells previously reported in CIN,⁹ since this lymphocyte subset normally expresses higher GGT levels than do the naïve CD45RA⁺ T-cells.¹⁰

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