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Circulating YKL-40 in patients with essential thrombocythemia and polycythemia vera treated with the novel histone deacetylase inhibitor vorinostat

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ABSTRACT

YKL-40 regulates vascular endothelial growth factors and induces tumor proliferation. We investigated YKL-40 before and after treatment with vorinostat in 31 polycythemia vera (PV) and 16 essential thrombocythemia (ET) patients. Baseline PV patient levels were 2 times higher than in healthy controls ($P < 0.0001$) and 1.7 times higher than in ET ($P = 0.02$). A significant correlation between YKL-40 at baseline and neutrophils, CRP, LDH, *JAK2*V617F and platelets in PV patients was observed, as well as a significantly greater reduction of YKL-40 levels in PV patients responding to therapy. YKL-40 might be a novel marker of disease burden and progression in myeloproliferative neoplasms.

1. Introduction

The Philadelphia-negative myeloproliferative neoplasms (MPNs), essential thrombocythemia (ET) polycythemia vera (PV), and primary myelofibrosis (PMF), arise due to acquired stem cell defects resulting in accumulation and proliferation of myeloid cells

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[1]. Transformations from ET to PV, and from both of these entities, to post-ET myelofibrosis (PET-MF) and post-PV myelofibrosis (PPV-MF) respectively, are common, and these observations have challenged the concept of these neoplasms as distinct disease entities. Evidence of progression has been demonstrated by an increasing *JAK2*V617F allele burden [2,3], and most recently gene expression profiling studies in ET, PV and PMF have suggested dysregulation of genes associated with immunity and inflammation [4–6]. Furthermore, progressive accumulation of reticulin, collagen and endothelial proliferation are major histopathological stromal changes in the bone marrow either present at diagnosis (PMF) or developing during the course of the disease (ET and PV) [7,8]. Both fibrogenesis and angiogenesis are considered to develop consequent to the intramedullary release of various growth factors from rapidly proliferating large and dysplastic megakaryocytes [7,8].

YKL-40 (chitinase-3-like protein 1) is expressed in various cell types including leukocytes [9], macrophages [10] and also in bone marrow megakaryocytes [11]. YKL-40 is encoded by the *CHI3L1* gene located on chromosome 1 and has been implicated in various inflammatory conditions, such as inflammatory bowel disease [12] and rheumatoid arthritis [13] as well as malignant disease including both solid tumors [14–16] and hematological malignancies [10,11,17,18]. YKL-40 regulates vascular endothelial growth factor [19,20] and ectopic expression of YKL-40 in cancer cells lead to tumor formation with an extensive angiogenic phenotype. Blockade of YKL-40 suppresses tumor angiogenesis both *in vitro* and *in vivo* [21]. The significance of YKL-40 in ET and PV has never been established and is the subject of this study.

In addition, histone deacetylase inhibitors (HDACi) have been demonstrated to induce tumor cells to undergo differentiation and apoptotic cell death [22–27] as well as inhibiting endothelial cell proliferation and angiogenesis *in vivo* [27–29]. A multicenter study of the efficacy and safety of vorinostat in ET and PV has most recently been published, showing that vorinostat reduces elevated cell counts in a proportion of the patients in concert with a decrease in spleen size [30]. Herein, we also report the effect of vorinostat on circulating YKL-40 levels.

2. Materials and methods

2.1. Study design

The study was an investigator-initiated, non-randomized, open-label phase II multicenter study. (EudraCT No. 2007-005306-49). Patients were included from 15 centers in Denmark, Sweden, The Netherlands and UK. Patients were not included if they had received interferon alpha, anagrelide or hydroxyurea (HU) within 1 week of day 1 or valproic acid within 28 days. Clinicohematological response assessment was per the European Leukemia Net (ELN) response criteria [31] and patients who obtained partial (PR) – or complete responses (CR) on vorinostat as monotherapy were defined as "responders". Assessment of splenomegaly was clinical by palpation. Fatigue, diarrhea and hair loss were graded by the use of Common Terminology Criteria for Adverse Events (CTCAE) version 3.0 and categorized as present or absent for this study.

2.2. Ethics

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008. Informed consent was obtained from all patients for being included in the study.

2.3. Vorinostat therapy

Vorinostat, 400 mg, was administered once daily. Concurrent administration of hydroxyurea (HU) was permitted under certain circumstances [30], but such patients were excluded from YKL-40 analysis in the present study in order to assess the effects of vorinostat as monotherapy.

2.4. Quantitative JAK2 analysis

DNA purification and real-time quantitative PCR (qPCR) were performed as described [30].

2.5. Serum YKL-40 and a normal reference range

Serum samples were stored at −80 °C until analyzed. Serum concentrations of YKL-40 were determined in duplicates using a sandwich-type enzyme-linked immunosorbent assay (ELISA) in accordance with the manufacturer's instruction (Quidel, Santa Clara, CA, USA). The intra-assay coefficient of variations (CV) was 5% and the inter-assay CV was <6%.

In a previous study the reference interval for plasma YKL-40 was determined in 3130 healthy subjects (1293 men, 1837 women) aged 21–84 years from the Danish general population, the Copenhagen City Heart Study. They had no known disease at time of blood sampling in 1991–1994 and remained healthy and alive during the 16 years follow-up period. The median plasma YKL-40 in these 3130 healthy subjects was 40 μg/l (min–max: 20–1098 μg/l; 5–95% percentile range, 20–116 μg/l) [32]. In order to convert plasma values to our serum a conversion factor of 1.4 was utilized [33]. Since YKL-40 increases with age Bojesen et al. have presented a formula for determining whether a patient has elevated YKL-40 [32]. We defined an elevated serum YKL-40 as higher than the 95% percentile in healthy controls.

2.6. Statistics

All variables were inspected for normality. Skewed variables were log transformed. Normally distributed variables were analyzed using parametric statistics including *t*-test for comparisons between groups, Pearson correlation for association between variables and linear regression for multiple analyses. These variables included YKL-40, hemoglobin, hematocrit, lactate dehydrogenase (LDH), platelet – and white blood cell counts. All these except for hemoglobin were log transformed. Variables without normal distribution even after log transformation were analyzed using non-parametric tests, Mann–Whitney test and Spearman correlation. These included *JAK2* mutation burden and C-reactive protein (CRP). Categorical variables were analyzed using Fisher's exact test. All tests were two-sided and *P*-values below 0.05 were considered significant. SPSS version 19.0 (IBM, Armonk, NY, USA) and R version 3.0.0 (R Foundation for Statistical Computing, Vienna, Austria) were used for all analyses.

3. Results

3.1. Baseline characteristics

Sixty-three patients (ET = 19, PV = 44) were enrolled between September 2008 and June 2010. From these patients YKL-40 serum samples were obtained from 31 PV and 16 ET patients at baseline and paired after 3 months of therapy for 23 PV and 16 ET patients. Missing follow-up samples were due to patient discontinuation from study. Quantitative *JAK2* analyses were also collected at baseline and after 3 months of therapy as well as other routine laboratory parameters (hemoglobin, hematocrit, platelets, leukocytes (total, neutrophils, monocytes), CRP, and LDH). Patient characteristics and laboratory data at baseline for the 47 patients are shown in Table 1.

When adjusting for age, YKL-40 levels were 1.2 times higher in the ET group than in healthy controls, however, this observation was not statistically significant, $P = 0.3$ (Fig. 1). We found YKL-40 levels in the PV group to be 2 times higher than in healthy controls ($P < 0.0001$) and 1.7 times higher than in the ET group ($P = 0.02$). Accordingly, only 25% (4/16) of ET patients exhibited elevated age-adjusted levels of YKL-40 at baseline whereas 52% (16/31) of PV patients did. We scrutinized existing clinical baseline data on all patients included in the study to examine whether these patients otherwise exhibited different clinical characteristics than were to be expected from their apparent diagnoses (Supplementary Table A.1).

Supplementary Table A.1 related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.leukres.2014.04.002.

Patients with elevated YKL-40 levels proved heterogeneous and consisted of both males and females, mutated and wild-type *JAK2*, and previously treated and newly diagnosed patients, however,

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