

# **Computational analysis of whole body CT documents a bone structure alteration in adult advanced chronic lymphocytic leukemia**

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## **Advances in Knowledge:**

1. Advanced chronic lymphocytic leukemia (ACLL) causes structural skeletal alterations, quantifiable by computational analysis of CT images, characterized by a mean 8% increase of trabecular bone prevalence (22% in the appendicular skeleton) with concomitant compact bone loss.
2. In ACLL, degree of bone erosion seems to predict a significantly lower two-year survival (18%) when compared to the one of the remaining patients (82%).
3. Radiological risk stratification has shown a better performance with respect to biological markers both at univariate ( $p=0.005$  vs  $0.073$ ) and multivariate ( $p=0.027$  vs  $0.092$ ) analysis.

## **Implications for Patient Care**

1. Radiological risk assessment could be used to predict disease aggressiveness and to better tailor patient-specific treatment protocols.

## **Abstract**

**Purpose:** We aimed to assess the presence of alteration of bone structure and bone marrow metabolism in adult patients with suspected advanced chronic lymphocytic leukemia (ACLL), using a computational prognostic model, based on computational analysis of PET/CT imaging..

**Materials and Methods:** In our retrospective study, all patients signed written informed consent as a requisite to undergo PET/CT examination. However, due to its observational nature, approval from the Ethical Committee was not deemed necessary.

Twenty-two treatment-naive CLL patients underwent PET/CT for disease progression. PET/CT images were analyzed using dedicated software, able to recognize an external 2-pixel bone ring whose Hounsfield coefficient served as cut off to recognize trabecular and compact bone. PET/CT data from 22 age and sex matched control patients were used as comparison. All data are reported as mean±standard deviation, Student's t-test, Log-rank or Cox proportional hazard model, were used as appropriate, considering a P value <0.05 as significant.

**Results:** Trabecular bone was expanded in ACLL patients and occupied a larger fraction of the skeleton with respect to controls, (39%±5% vs 31%±7%, mean±standard deviation, i.e. 32/81 vs 27/86 ml/Kg of ideal body weight respectively, p<0.001). After stratification according to median value, patients with a ratio trabecular/skeletal bone volume >37.3% showed an actuarial two-year survival of 18% compared with 82% for those with ratio<37.3% (p<0.001), independently form age, gender, biological markers and disease duration.

**Conclusions:** These data suggest that computational assessment of skeletal alterations might represent a new window to predict the clinical course of the disease.

**Keywords:** Image Analysis, Bone Marrow, Skeletal Structure, ACLL, PET/CT

## Introduction

Chronic lymphocytic leukemia (CLL) can display a largely variable clinical course. Being generally regarded as a low-grade hematologic neoplasia, it can indeed remain indolent for years and decades, requiring no treatment. However, at a certain point of its natural history, it will progress to its accelerated phase, defined as advanced chronic lymphocytic leukemia (ACLL). Progression to this stage is linked to the evolution of the disease to a less differentiated, more aggressive and less chemo-sensitive clone; in this context, risk stratification gets extremely complex, as clinical staging and biochemical marker that had been used at diagnosis tend to lose their relevance [1-2].

Recent experimental research indicates a relevant role for the interaction between neoplastic and stromal cells within the hematopoietic niche. In mouse models, leukemia causes a measurable bone loss [3] whose role in disease evolution has been independently proven [4-5]. Should this concept extend to the clinical setting, it might improve our understanding the mechanisms underlying leukemia progression and thus in our capability to stratify patients risk.

Unfortunately, reliable methods to characterize whole body skeleton in adult humans are still missing. In fact, although the potential of magnetic resonance imaging in evaluating bone marrow composition [6-8] is well recognized, the complexity of acquisition procedure and the influence of signal distortion limited its use to specific bone districts. To extend the evaluation of bone marrow to the whole skeleton, a software approach to PET/CT images able to computationally integrate anatomical and functional information was recently developed [9]. By exploiting the attenuation coefficient of compact bone, this method allows an accurate recognition and estimation of the different bone compartments, as well as the extraction of functional data within the trabecular bone volume throughout the whole skeleton as well as in specific bone districts.

Our study thus aimed to assess the presence of alteration of bone structure and bone marrow metabolism in a cohort of adult patients with suspected ACLL, using a computational prognostic model, based on computational analysis of PET/CT imaging.

## **Material and Methods**

### *Study sample, inclusion criteria and biological risk assessment*

Our retrospective study included 22 consecutive inpatients (mean age  $56\pm 17$ , range 18-81), admitted to our hospital because of suspected A CLL. As shown in Table 1, the group included 12 men, (mean age  $50\pm 18$ , range 29-81) and 10 women (mean age  $60\pm 16$ , range 18-71) (mean $\pm$ standard deviation).

All patients were submitted to whole-body FDG PET/CT imaging to rule out Richter's transformation [10]. According to our standard procedure, all patients signed a written informed consent form, encompassing the use of anonymized data for retrospective research purpose, before PET/CT examination.

Exclusion criteria included previous CLL therapy, bone marrow transplant, radio or chemotherapy, as well as previous corticosteroid therapy and any evidence of active or recent infection or inflammation.

We compared these patients with matched healthy controls, randomly selected from a published normalcy database [9]. Briefly, this database includes patients submitted to PET/CT scan as a long term control of completely-excised first stage melanoma, never submitted to any chemo- or radiotherapy and negative at a two-year follow-up. This case-control selection matched age, sex and menopausal status in women; mean age was  $55\pm 18$  years comprising 12 men, (mean age  $58\pm 19$ , range 22-89) and 10 women (mean age  $52\pm 14$ , range 28-69).

Risk stratification had been performed at diagnosis according to current guidelines [11-14]: high risk was defined in the absence of IgH mutation, in the case of deletion of the chromosome 17 short arm or by ZAP70/CD38 over-expression. T-cell CLL (n=1) was defined as high risk.

In our study, off-line computational analysis was applied to PET/CT data collected in the clinical workup and did not influence patient care in any way. Accordingly, due to this observational nature, and in accordance with the Italian laws, approval from the Institutional Review Board was not deemed necessary.

### *PET-CT acquisition and reconstruction*

After a minimum of 12 hours fasting, serum glucose was assessed to ensure glucose level  $\leq 1$  g/l. Weight and height of all patients was measured. The latter variable was used to calculate ideal body weight according to the formulation of Robinson et al [15]. A bolus injection of FDG was then performed (4.8-5.2 MBq per kilogram of body weight) while patients were lying in supine position in a quiet room and instructed not to move or talk. PET imaging started 60 to 75 minutes after tracer administration and was performed in 3D mode, using an integrated PET/CT scanner, (Hirez; Siemens Medical Solutions, Knoxville TN, USA). The whole body was scanned from vertex to toes in an “arms down” position. PET raw data were reconstructed by means of ordered subset expectation maximization (OSEM, 3 iterations, 16 subsets) and attenuation correction was performed using CT data. The transaxial field of view and pixel size of the reconstructed PET images were 58.5 cm and 4.57 mm, respectively, with a matrix size of 128×128. As per standard PET/CT imaging protocol, 16-detector row helical CT scan was performed with non-diagnostic current and voltage settings, with a gantry rotation speed of 0.5 s and table speed of 24 mm per gantry rotation. No contrast medium was injected. The entire CT dataset was fused with the 3-dimensional PET images using an integrated software interface (Syngo; Siemens Erlangen, Germany).

### *Image analysis*

Image analysis was performed according to the previously validated method [9]. The algorithm identifies the skeleton on CT images by assuming that compact bone is the structure with the highest X-ray attenuation coefficient in the human body. Once identified the skeletal border, the program starts the thresholding algorithm, which samples a 2-voxel-thick layer and computes its average Hounsfield value. Thereafter, all skeletal voxels having attenuation coefficient equal or above this value are considered as compact bone, the remainings trabecular bone. The software output is the extraction and 3D-representation of three different volumes: skeletal volume, compact bone volume and trabecular bone volume. Then, two nuclear physicians (SM and MM, with a 5-years experience

in PET/CT images reporting) manually proceeded to exclude the spinal cord and the non-skeletal calcified regions.

Trabecular bone volume is then voxel-wise multiplied against the PET co-registered data to extract and represent bone marrow metabolic activity. The skull is intentionally excluded from the analysis, since brain radioactivity spillover prevents an accurate assessment of bone marrow FDG uptake in this region. The computation of whole skeletal volumes was completed by a further segmental analysis: vertebrae and sternum accounted for the axial skeleton; humeral, femoral and tibial/fibular shafts for the appendicular skeleton.

All volumetric data were normalized for ideal body weight. All trabecular bone volume voxels were subdivided into active or inactive bone marrow according to previously published statistical standards [9]. This criterion assumes that lumbar and thoracic vertebrae are almost fully occupied by active bone marrow. In this district, average standardized uptake value (SUV) was found to be  $2.01 \pm 0.36$  in a population of 102 normal subjects. Assuming a normal distribution of these values, values of SUV located 2.5 standard deviations below the average (i.e. less than 1.11) were considered as idle, fatty bone marrow. Active bone marrow was defined as all intraosseous voxels, in all skeletal segments, with FDG uptake greater than 1.11.

### *Statistical analysis*

All data are reported as mean  $\pm$  standard deviation. Unpaired or paired t-tests were used to compare data in different groups or in the same group, as appropriate. Linear regression analysis was performed using the least squares method. Survival curves were plotted and survival rates were calculated using the Kaplan-Meier method and Log-rank  $\chi^2$  test. In multivariate analysis, backward stepwise regression analysis was carried out using a Cox proportional hazards model. A *P* value < 0.05 was considered statistically significant. All statistical analyses were carried out using SPSS software version 20 (SPSS Inc., Chicago, IL).

## Results

### *Clinical findings*

In the whole study cohort, hospital admission for suspected ACLL occurred  $27\pm 25$  months after diagnosis of CLL. At the time of hospital admission, anemia was present in 8/22 (36%) patients. Platelet count was relatively preserved, while leukocytosis was documented in 4 patients (18%). Seven patients (32%) had lactate dehydrogenase  $> 1.5$  times normal level. Lymph node involvement, subsequently confirmed at FDG imaging, was observed in 8 patients (36%). Patients' clinical features are summarized in Table 1.

After PET/CT scans, follow up was carried out for a median duration of 24 months (range 6-36).

During this period, 11 patients (50%) died because of disease progression (Table 1). Age, gender, hemoglobin level, blood elements counts and LDH levels were similar in the two subgroups.

Similarly, both these subgroups had a comparable progression free survival between CLL diagnosis and ACLL onset (Table 1).

### *Image analysis: morphological findings*

Whole body skeletal volume was similar in leukemic patients and in control subjects ( $81\pm 15$  vs  $85\pm 12$  ml/Kg ideal body weight, respectively,  $p=0.126$ , ns, Figure 1A). In contrast, ACLL was associated with a significant trabecular bone volume enlargement ( $32\pm 8$  vs  $27\pm 8$  ml/Kg ideal body weight, respectively,  $p=0.037$ , Figure 1A). The expansion of the intraosseous space was paralleled by a concomitant decrease of CBV in patients with respect to controls ( $49\pm 8$  vs  $58\pm 9$  ml/Kg ideal body weight, respectively,  $p<0.001$ , Figure 1 A).

Structural bone alterations barely involved the axial skeleton (Figure 1B), while they were evident in the appendicular bones (Figure 1C). In fact, composition of long bone diaphysis was profoundly altered in ACLL with a clear enlargement of trabecular bone volume in patients compared to controls ( $7.2\pm 1.6$  vs  $3.7\pm 1.2$  ml/Kg ideal body weight, respectively,  $p<0.001$ ), facing a marked loss of

compact bone volume ( $10.6 \pm 1.5$  vs  $17.9 \pm 2.9$  ml/Kg ideal body weight, respectively,  $p < 0.001$ ) (Figure 1 C, D, E).

Skeletal alterations induced by leukemia were best appreciated by analyzing the ratio between trabecular bone, as a measure of the space occupied by bone marrow, and total skeletal volume. In fact, trabecular bone volume/skeletal volume ratio was higher in the leukemic group than in control subjects ( $39\% \pm 5\%$  vs  $31\% \pm 7\%$ , or  $32/81$  vs  $27/86$  ml/Kg of ideal body weight, respectively,  $p < 0.001$ ) (Figure 1 F). This difference was even more marked in the appendicular compartments ( $40\% \pm 4\%$  vs  $18\% \pm 5\%$ , or  $7.2/17.9$  vs  $3.7/19.6$  ml/Kg of ideal body weight in leukemics and controls, respectively,  $p < 0.001$ , Figure 1 F).

#### *Image Analysis: Metabolic Findings*

Average SUV of tissue residing within trabecular bone volume was similar in patients and in controls ( $1.13 \pm 0.33$  vs  $1.0 \pm 0.2$ , respectively,  $p = 0.08$ ). Volumetric analysis of bone marrow distribution showed that extension of the metabolically active bone marrow (defined as voxels with  $SUV \geq 1.11$ ) was similar in patients and in controls, both in the whole skeleton ( $10.2 \pm 4.5$  vs  $9.4 \pm 2.5$  ml/Kg ideal body weight, respectively,  $p = 0.35$  and in the axial compartments ( $4.1 \pm 1.2$  vs  $3.7 \pm 0.8$  ml/Kg ideal body weight, respectively,  $p = 0.09$  Figure 1G).

Conversely, active bone marrow was more extended in appendicular segments of leukemic patients than control subjects ( $0.8 \pm 0.2$  vs  $0.3 \pm 0.1$  ml/Kg ideal body weight, respectively,  $p = 0.015$ , Figure 1G). However, the average gap in the active bone marrow extension between patients and controls (0.5 ml per Kg of ideal body weight) was largely inferior with respect to the average difference in trabecular bone volume of the corresponding region (3.5 ml/Kg ideal body weight).

#### *Image analysis: prognosis*

Degree of skeletal structure alteration displayed a relevant prognostic significance. In fact, the eleven non-survivor patients showed a larger trabecular bone volume expansion compared to survivors

(Figure 2A and 2B), as demonstrated by a significant increase in trabecular bone volume /skeletal volume ratio ( $41.5\pm 4.7\%$  vs  $35\pm 3.3\%$ , or  $36/87$  vs  $26/75$  ml/Kg of ideal body weight, in non-survivors and in survivors, respectively,  $p=0.007$ , Figure 2 C). Again, this difference was trivial in axial bones and particularly pronounced in long bone shafts ( $43\pm 6\%$  vs  $30\pm 4\%$ , or  $33/76$  vs  $18/64$  ml/Kg of ideal body weight, in non-survivors and in survivors, respectively,  $p=0.004$ , Figure 2 C). Actually, the degree of trabecular bone volume expansion was found to be dependent from the time elapsed from CLL diagnosis ( $r=0.56$ ,  $p=0.004$ ). However, a significant trabecular bone volume difference between survivors and non-survivors was maintained at any time point, regardless the length of the interval between CLL diagnosis and disease progression (Figure 2D).

Due to the limited patients number, the prognostic role of bone structure alteration was tested by dividing the whole study cohort into two groups, on the basis of the median trabecular bone volume / skeletal volume ratio (37.3%). Kaplan-Meier analysis showed that large degree of bone erosion was associated with increased mortality in the subsequent two years (Log Rank,  $p<0.001$ , Figure 2E), while the association between biological risk characterization and overall survival was not as robust as the one provided by imaging findings (Figure 2F).

At univariate analysis, the only predictive variables for subsequent death were bone erosion, measured at imaging by trabecular/skeletal volume ratio, and biological characterization of neoplastic cells (Table 3). Moreover, multivariate Cox models documented the independent nature of imaging risk assessment ruling out any significant contribution of age, gender, neoplastic clone phenotype, lymph node involvement and CLL duration (Table2). As a consequence, the hazard ratio associated with trabecular bone volume / skeletal volume  $>37.3\%$  was comprised between 19.8 and 22.4 in all multivariate models and was practically identical to the value estimated in the univariate analysis (20).

## **Discussion**

Our retrospective observational study documents the impact of ACLL on skeletal structure, causing a significant expansion of the intraosseous volume at the expense of cortical thinning. This phenomenon is detectable and quantifiable at computational analysis. Actually, when considering the

appendicular segments, the skeletal alterations are evident even at a qualitative, non-computational, inspection of CT slices. The degree of trabecular bone expansion and compact bone loss appears to be a powerful predictor of the two-year-mortality in these ACLL patients. The prognostic index provided by imaging is independent from biological risk stratification, extension of neoplastic burden, patient age and gender, duration of the chronic disease phase and biological characterization of neoplastic clone. **These results indicate that ACLL is associated with a loss of compact bone whose evaluation might represent a new window to predict patient outcome.**

Our study documents that ACLL is associated with an array of skeletal alterations, which cannot be attributed to the confounding interference of treatment, since the study only included patients that had not been previously treated for CLL. These skeletal alterations appear to be related to a primary effect of the disease, reproducing in the clinical setting the leukemia-mediated osteoblastic impairment, which, according to recent experimental evidence, has a relatively early onset in the course of leukemia pathogenesis [3].

The limited bone erosion observed in axial skeleton apparently disagrees with the notion that trabecular bone density is actually hampered in vertebral bodies of experimental leukemia and myeloma [3, 16]. However, this observation probably reflects the limited spatial resolution of our low dose attenuation correction CT scanning protocol and the consequent influence of partial volume averaging effect [17]. On the other hand, cortical thinning was well evident in long bone shafts and, while it paralleled the expansion of intraosseous space, it largely exceeded the minimal expansion of the metabolically active bone marrow quota, as documented by the analysis of the co-registered PET maps. Accordingly, these data suggest that ACLL affects bone structure with further paracrine-endocrine mechanisms besides the direct effect of leukemic clone expansion [18-21]. In this line, the skeletal alteration might represent a useful index to define disease biology and to characterize the pathways underlying its progression.

The evidence of altered skeletal composition suggests a possible prognostic relevance in adult A CLL patients: greater degree of cortical loss, documented by increased values of trabecular bone volume/skeletal volume ratio, portends a more aggressive disease, with higher mortality rate. This finding, albeit somewhat unexplored in the clinical field, agrees with current models of disease progression derived from experimental models of leukemia. In fact, cells of the mesenchymal-osteoblastic lineage play an essential role in the regulation of normal hematopoietic stem cells [4-5, 22]. On the other hand, specific disruptions of the osteoblastic compartment can result in myeloproliferative disorders even without genetic manipulation of the hematopoietic system [23]. Accordingly, our data confirm that leukemia progression and A CLL onset depend upon interactions among all the components of the hematopoietic niche.

There are some limitations that need to be mentioned. First, our study was limited by its retrospective nature. Second, the clinical role of quantitative image analysis in A CLL patients needs to be determined by further studies. In fact, the studied sample was relatively small, due to the selection criteria that only included patients with suspected disease progression, evaluated after a largely variable period of indolent course (from 3 to 83 months). In this setting, prognostic value of biological characterization of neoplastic clone was relatively low, confirming the notion that the highest value of the conventional staging systems applies to the early phases of CLL and becomes less useful as the disease progresses [1-2, 25]. Third, in accordance with the selection criteria, patients with indolent and stable CLL were not evaluated, since performing PET/CT imaging in CLL is only justified whenever there is founded clinical suspicion for disease progression or complications [10,28]. Consequently, our analysis does not permit to define presence, degree and progression rate of bone loss over the entire course of the chronic phase of the disease. Actually, trabecular bone enlargement was at least partially correlated with duration of chronic phase. However, this alteration was consistently more pronounced in the high-risk subgroup at any time point after CLL diagnosis.

Further investigations, with a larger study sample, are needed to elucidate molecular pathways underlying the interference between neoplastic clone and bone homeostasis as well as to better define the clinical role of this information.

In conclusion, our quantitative analysis represents an imaging-based approach that is potentially able to study the interaction between the leukemic clone and the intraosseous environment. Whether confirmed in larger studies, it might help in A CLL risk stratification. Similarly, its application in the course of experimental treatments might represent a new window to understand the mechanisms underlying their potential effectiveness.

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