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ORIGINAL ARTICLE

# Oxidative stress accelerates spontaneous apoptosis of B-chronic lymphocytic leukemia lymphocytes

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## Summary

***Purpose:*** *B-chronic lymphocytic leukemia (B-CLL) is characterized by the progressive accumulation of small immature B lymphocytes which do not undergo apoptosis due to an underlying defect. One potential mechanism of defective apoptosis could be irregular oxidative stress. The goal of our investigation was to determine in vitro production of oxidative stress markers by lymphocytes of B-CLL patients.*

***Patients and methods:*** *30 untreated stage A B-CLL patients, as well as 20 stage B and C patients and 30 healthy volunteers as a control group were examined. Nitric oxide (NO), superoxide anion, hydrogen peroxide and malondialdehyde (MDA) were measured by spectrophotometry in supernatants of lymphocytes cultures of all 3 investigational groups. The method applied for detecting apoptosis was fluorescence microscopic analysis using acridine orange/ethidium bromide (AO/EB) double staining.*

***Results:*** *In vitro lymphocyte production of superoxide anion, hydrogen peroxide and MDA was increased in B-CLL patients, while there were no statistical significantly differences of NO production among the tested groups. Compared with the spontaneous apoptosis observed in control subjects lymphocytes, B-CLL lymphocytes showed increased percentages of apoptotic cells after incubation for 24 h. Disease progression was not followed with significant differences in spontaneous apoptosis of B-CLL lymphocytes.*

***Conclusion:*** *This intensive oxidative stress markers production in cultures of B-CLL lymphocytes could be one of the potential mechanisms in the pathogenesis of abnormal apoptosis.*

**Key words:** apoptosis, chronic lymphocytic leukemia, hydrogen peroxide, malondialdehyde, nitric oxide, superoxide anion

## Introduction

B-CLL is characterized by the accumulation of monoclonal, functionally immature $CD5^+$ B lymphocytes [1]. The progressive increase of lymphocyte count coupled with the very low proportion of proliferating cells has led to the notion that B-CLL may be originated by defective apoptosis [2].

Apoptosis is the physiological process accompanied with many morphological and biochemical changes whereby most cells, including B lymphocytes, are eliminated; this process leads to homeostasis. It is well known that the majority of B-CLL cells have a long lifespan *in vivo*, but such cells rapidly undergo spontaneous apoptosis *in vitro* [3,4]. *In vitro* poor survival may be a result of lack of accessible growth factors because the addition of several cytokines, including interleukin-4, interleukin-6, interleukin-8 and interferons α and γ, has been shown to promote CLL cells survival *in vitro* [5]. The role of oxidative stress in the pathogenesis of this disease is poorly understood and a matter of interest.

Oxidative stress, a well known phenomenon characterized with overproduction of chemically active free

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