Monoclonal B-cell lymphocytosis and prostate cancer: incidence and effects of radiotherapy

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ABSTRACT

Monoclonal B-cells lymphocytosis (MBL) is a benign condition that may precede chronic lymphocytic leukemia (CLL), not rarely present in peripheral blood of healthy elderly people, among which there is also a male prevalence. Though CLL has been associated with various types of solid tumors, including prostate cancer (PC), no data exist about the relationship between PC and MBL. We studied the frequency of CLL-like MBL clones in a group of 48 patients affected by PC and followed them during and after whole-pelvis radiotherapy (WPRT) treatment. We found four MBL clones (8.3%), two of which (4.2%) had a B-cell clonal count >1000 cells/µL ('clinical MBL'). A single case (1.8%) of 'low-count' MBL occurred in a control group of 54 healthy males. Notably, normal B-lymphocytes were consistently affected by WPRT, while MBL clones were less radiosensitive. Our results suggest a possible association between 'clinical' MBL and PC and show a different impact of the radiation on monoclonal respect to normal B-cells, which could also imply a greater risk of clonal transformation.

Significance of this study

What is already known about this subject?
- Monoclonal B-cells lymphocytosis (MBL) is a benign condition that may precede chronic lymphocytic leukemia (CLL).
- CLL has been associated with various types of solid tumors, including prostate cancer (PC).
- No data exist about the relationship between PC and MBL.

What are the new findings?
- We found four MBL clones (8.3%) in the group of patients affected by PC.
- Two of them (4.2%) had a B-cell clonal count >1000 cells/µL.
- Normal B-lymphocytes were consistently affected by whole-pelvis radiotherapy, while MBL clones were less radiosensitive.

How might these results change the focus of research or clinical practice?
- Our results suggest a possible association between 'clinical' MBL and PC that warrant further investigation.

INTRODUCTION

Monoclonal B-cell lymphocytosis (MBL) is a recently recognized entity characterized by the presence, in the peripheral blood, of a clonal B-cell population <5000/µL, in the absence of any type of clinical features.[1] MBL clones may have (i) typical chronic lymphocytic leukemia (CLL-like) phenotype (CD5+, CD19+, CD23+, CD20 dim); (ii) atypical CLL phenotype (CD5+, CD19+, CD23− or CD20 bright); and (iii) non-CLL phenotype (CD5−).[1] MBL can be also distinguished in 'low-count' (<500/µL) and 'high-count' (>500/µL) subtypes. High-count MBL frequently shows typical CLL phenotypic/genetic features and requires adequate follow-up in order to detect its possible evolution into symptomatic CLL.[2] MBL showing a clonal B-cell count >1000–1500/µL is usually defined 'clinical' MBL.[3]

Using highly sensitive (ie, >6 colors and >500,000 events) flow cytometry approaches, MBL clones have been found at a frequency of 7%–12% in healthy subjects, showing, however, very low median counts of clonal B-cell (10–170/µL), with only 0.14% being clinical MBL.[4 5]

Though several studies have described the association between CLL and various types of neoplastic disorders,[6–8] only few data exist about the risk of non-hematological cancer in individuals with MBL; in particular, no association between MBL and prostate cancer (PC) has been so far reported.[9]

After the occasional observation of an apparently increased MBL incidence at baseline in a cohort of patients with PC originally studied to detect lymphocyte abnormalities possibly induced by radiotherapy (RT),[10] we investigated here prospectively, with 6 colors and 100,000 events flow cytometry approaches, the frequency of CLL-like MBL clones in patients affected by PC treated with whole-pelvis radiotherapy (WPRT) compared with healthy males of similar ages. When found, we followed the clones during the 6 months after WPRT.

PATIENTS AND METHODS

We prospectively analyzed 48 patients affected by PC (mean age 72.9 years, SD ±8 years), naïve

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Table 1 Absolute levels of WBC and lymphocytes in tested patients and normal controls

	Median WBC/µL (range)	Median lymphocytes/µL (range)	Median B-cells/µL (range)	Absolute MBL clone values/µL
Prostate cancer (n. 44)	7100 (3340–13770)	2150 (800–4000)	136 (26-439)	
Prostate cancer with MBL clone (n. 4)	8059 (5800–12900)	2900 (1700–4900)	949 (380–1984)	1970, 1254, 294, 85
Healthy controls (n. 54)	7240 (4090–12380)	2100 (800–5100)	160 (28–484)	8

MBL, monoclonal B-cell lymphocytosis; WBC, white blood cells.

for chemotherapy, 24 of whom were previously treated with hormone therapy. All patients were planned to receive WPRT with radical (n. 33) or salvage (n. 15) intent. The irradiation techniques used were conformal (n. 13) or Rapid Arc (n. 35), respectively. Fifty-four healthy males (mean age 71 years, SD±8.5 years) represented the control group.

Immunophenotypic analysis of peripheral blood lymphocytes was performed by BD FacsCanto II flow cytometer using the FacsDiva software (BD Biosciences) and a 5–6 colors approach and the following antibody combinations: CD19 FITC/CD5 PE/CD45 PerCP/CD20 PE-Cy7/CD23 APC; Kappa FITC/Lambda PE/CD19 PerCP-Cy5.5/CD20 PE-Cy7/CD5 APC/CD45 APC-Cy7 (reagents purchased from BD Biosciences). For each sample, 100,000 events were collected. CD45+ lymphocytes were gated on CD45 versus SSC dot plot, then B-cells were isolated by gating on CD19 and finally the CD19+CD5+CD20 dim population was analyzed for light chain clonality and CD23 expression. The minimum number of events considered as a monoclonal B-cell cluster was 50.[4]

Evaluation times were: before RT (t0), end of RT (t1), 3 months after RT (t2), and 6 months after RT (t3).

Complete blood counts were obtained with a Beckman Coulter DXH800. All patients had a normal blood cell count, with no evidence of relative or absolute lymphocytosis.

For the literature review, the search was performed using PubMed from January 2001 through August 2018, looking for studies or case reports of the association of MBL with PC.

Statistical analysis of the differences among groups was performed by using the Fisher's exact test. The threshold for statistical significance was $p<0.05$.

RESULTS

In the PC group, we identified four MBL (8.3%), two of which were 'high count/clinical' MBL (4.2%) as the monoclonal B-cell count was 1254 and 1970/µL, respectively. The other two patients had a monoclonal B-cell count of 294 and 85/µL. By contrast, in healthy subjects' group, only one 'low count/non-clinical' MBL was found (1.8%), showing a very low number of clonal lymphocytes (8/µL). Such a difference was, anyway, not statistically significant ($p=0.2$).

Median and ranges of absolute counts of white blood cells (WBCs), total lymphocytes and B-cells, as well as absolute single values of MBL clones (before RT) are reported in table 1. In figures 1 and 2, we reported the WPRT modulation of both MBL clones and normal B-cells, respectively, in the four patients with PC. Patients 1, 2 and 4 underwent Rapid Arc irradiation technique, the first two with 3.1 Gy fractionation and prescription dose of 62 Gy; the third with 2.7 Gy fractionation and prescription dose of 67.5 Gy. Patient 3 underwent conformal irradiation technique with 1.8–2 Gy fractionation and prescription dose of 73 Gy.

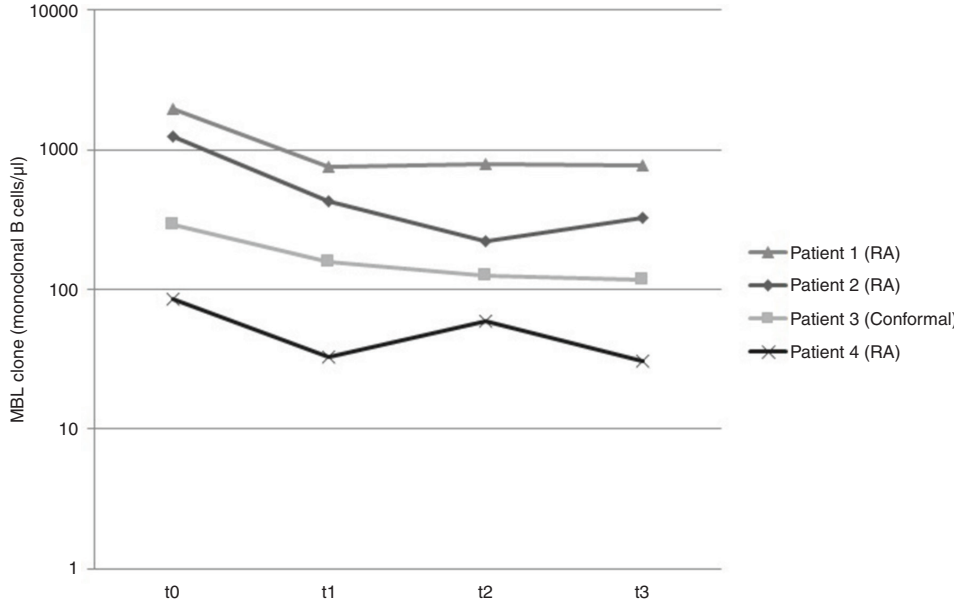

Figure 1 Evolution of the four monoclonal B-cell lymphocytosis (MBL) during and after radiotherapy (RT). RA, Rapid Arc technique; t0, before RT; t1, end of RT; t2, 3 months after RT; t3, 6 months after RT.

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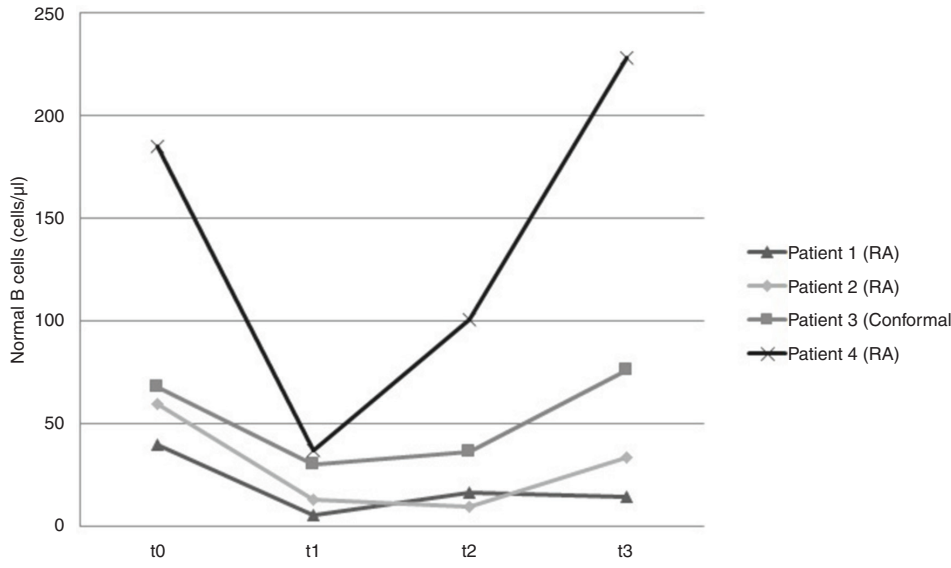

Figure 2 Evolution of the normal B-cells during and after radiotherapy (RT) in the four patients with concomitant monoclonal B-cell lymphocytosis and prostate cancer. RA, Rapid Arc technique; t0, before RT; t1, end of RT; t2, 3 months after RT; t3, 6 months after RT.

In these four patients, at the time point t1 (end of RT), the monoclonal B-cells and the normal B-cells were 38% (range 34%–53%) and 21% (range 17%–44%) of the basal levels, respectively. At the time point t3 (6 months after RT), the monoclonal B-cells and the normal B-cells were 37% (range 26%–39%) and 84% (range 37%–123%) of the basal levels, respectively. In particular, two patients (3 and 4 in figure 2) recovered the initial levels of normal B-cells and two did not (patients 1 and 2 in figure 2). The grade of the recovery at t3 was inversely correlated to the dimension of the MBL clone at the time point t0 (before RT), that is, bigger was the clone, smaller was the recovery of the normal B-cells.

Literature review

Regarding a possible association between MBL and PC, we did not find any specific report in the literature. A large study described an increased risk of cancer of the breast, lung and gastrointestinal tract (but not PC), developed in a period of 4.6 years median follow-up, in patients with high count MBL compared with controls.[9]

DISCUSSION

The association of CLL and PC is quite frequently reported in the literature. Tsimberidou *et al* found that patients with CLL or small lymphocytic lymphoma have more than twice the risk of developing a second cancer, of which prostate is 13%.[8] The study of Royle *et al*[11] also showed an increased risk of second cancer for patients with CLL, in terms of standardized incidence ratio (SIR), particularly melanoma (SIR=7.40), lip (SIR=6.26), but also prostate (SIR=1.94). In a population of untreated, long-term survivors of CLL, PC was the second most common other cancer, after non-melanoma skin tumors.[12] An increased risk for metachronous CLL was found in PC (SIR 1.3; 95% CI 1.1 to 1.5) and squamous cell skin cancer survivors (SIR 2.3; 95% CI 1.9 to 2.7).[13] An unusual histological finding of PC with concomitant prostatic localization of CLL has been also described.[14 15] Finally, we found also a report of a patient with an untreated CLL who developed acute promyelocytic leukemia 2 years after radiotherapy for PC.[16]

Our study is, to the best of our knowledge, the first investigation of MBL in patients affected by PC planned to be treated with WPRT. Overall, we found in these patients a 4.5-fold increased incidence of MBL, respect to normal controls. This result did not reach a statistical significance, likely due to the very low number of cases and events. However, it is interesting to note that the only case found in the control group was a 'very low count/non-clinical MBL' of only 8 cells/µL, which is below the median number of clonal B-cell count observed in healthy blood donors investigated with a higher sensitivity cytofluorimetric approach.[4] Indeed, if we did not consider this case as a 'true' MBL, the difference doubled and approached statistical significance (p=0.054).

Among lymphocyte subpopulations, B-cells are the most radiosensitive[17 18] as they can undergo radiation-induced apoptosis.[19] We followed the four clones of patients with PC during and after RT. Hematological toxicity is, in fact, a well-documented effect of WPRT,[20–22] in particular lymphopenia.[23–25]

In our patients, normal B-lymphocytes were consistently affected by WPRT as they rapidly decreased at the end of RT (median of reduction: 79%) as also reported in other studies in which the effects of RT on the different lymphocyte subpopulations were analyzed in patients affected by breast cancer, seminoma testis or PC.[17–19 26] Nevertheless, as shown in figure 2, they also tended to reincrease 3–6 months later, possibly by B-cell precursors which originate from the non-irradiated bone marrow.[19] By contrast, clonal B-cells appeared to be less radiosensitive as they did not show significant modifications in their number (figure 1), despite a limited decrease was anyway observed immediately after WPRT (median of reduction: 62%). Such modifications were apparently independent on the RT technique employed, though the very low number of patients do not allow any conclusion in this setting.

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The different effect of the radiation on the clone respect to the normal B lymphocyte here described, and the consequent possibility of a greater risk of clonal transformation, prompted us to monitor the four patients during time. After a follow-up of 24 months, the monoclonal B-cells remained under the original values, and the patients did not develop a CLL (data not shown).

In summary, the preliminary results of our prospective study, performed using a routine flow cytometric approach, highlight a possible association between (clinical?) MBL and PC, never described before and probably warranting further investigation in a larger number of patients.

Moreover, we described the different effect of the radiation on the MBL-clone with respect to the normal B-lymphocytes, whose potential clinical implications (clonal transformation?) remain to be defined.

Contributors FD designed the research study, analysed the data and wrote the paper. LR designed the research study and enrolled the patients. LV and TS performed flow cytometric analysis. GC and GD analyzed the data. VF designed the research study. PM designed the research study, analysed the data and wrote the paper.

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