Published in final edited form as:
Int J Cancer. 2009 February 15; 124(4): 958–963. doi:10.1002/ijc.23958.

Prevalence and frequency of circulating t(14;18)-MBR translocation carrying cells in healthy individuals

Frank Schüler[1], **Lars Dölken**[1], **Carsten Hirt**[1], **Thomas Kiefer**[1], **Tobias Berg**[1], **Gerhard Fusch**[2], **K. Weitmann**[3], **W. Hoffmann**[3], **Christoph Fusch**[2], **Siegfried Janz**[4], **Charles S. Rabkin**[5], and **Gottfried Dölken**[1,*]

[1]Department of Hematology and Oncology, Ernst-Moritz-Arndt-University Greifswald, Sauerbruchstraße, Greifswald, Germany

[2]Department of Pediatrics, Ernst-Moritz-Arndt-University Greifswald, Sauerbruchstraße, Greifswald, Germany

[3]Institute for Community Medicine, Ernst-Moritz-Arndt-University Greifswald, Sauerbruchstraße, Greifswald, Germany

[4]Department of Pathology, The University of Iowa Roy J. and Lucille A. Carver College of Medicine, Iowa City, IA

[5]Viral Epidemiology Branch, National Cancer Institute, Bethesda, MD

Abstract

The t(14;18) translocation is a common genetic aberration that can be seen as an early step in pathogenesis of follicular lymphoma (FL). The significance of low level circulating t(14;18)-positive cells in healthy individuals as clonal lymphoma precursors or indicators of risk is still unclear. We determined the age dependent prevalence and frequency of BCL2/IgH rearrangements in 715 healthy individuals ranging from newborns to octo- and nonagenarians. These results were compared with number of circulating t(14;18)-positive cells in 108 FL patients at initial presentation. The overall prevalence of BCL2/IgH junctions in this large sample was 46% (327/715). However, there was a striking dependence upon age. Specifically, among individuals up to 10 years old, none had detectable circulating t(14;18)-positive cells. In the age groups representing 10–50 years old, we found a steady elevation in the prevalence of BCL2/IgH junctions up to a prevalence of 66%. Further increases of the prevalence in individuals older than 50 years were not seen. The mean frequency of BCL2/IgH junctions in healthy individuals ≥40 years ($18–26 \times 10^{-6}$) was significantly higher than in younger subjects ($7–9 \times 10^{-6}$). Four percent (31/715) of individuals carried more than one t(14;18)-positive cell per 25,000 peripheral blood mononuclear cells (PBMNCs). In comparison, 108 stage III/IV FL patients had a median number of circulating t(14;18)-positive malignant FL cells of about 9200/1 million PBMNCs (range 7–1,000,000). These findings will further improve the understanding of the relevance of t(14;18)-

[*]**Correspondence to:** Klinik und Poliklinik für Innere Medizin C, Hämatologie und Onkologie–Transplantationszentrum, Ernst-Moritz-Arndt-Universität Greifswald, Sauerbruchstraße, D-17475 Greifswald, Germany. Fax: +49-3834-866713. doelken@uni-greifswald.de.

Additional Supporting Information may be found in the online version of this article.

positive cells in healthy individuals as a risk marker toward the development into lymphoma precursors.

Keywords

t(14;18)-translocation; BCL2; quantitative real-time PCR; healthy individuals; follicular lymphoma

The t(14;18)-translocation is one of the most common genetic aberrations in lymphoid malignancies. It involves the immunoglobulin heavy chain (IgH) locus joining-genes (J_H) on chromosome 14q32 and the BCL2 gene on chromosome 18q21 and can be detected cytogenetically in about 90% of follicular lymphomas (FL) and in 20% of diffuse large B-cell lymphomas.[1–3] Although the resulting overexpression of the proto-oncogene BCL2 can be envisaged to represent one of the first steps to the pathogenesis of FL, the acquisition of additional genetic aberrations is necessary for the progression to malignant follicular lymphoma.[4–9]

Given these findings, it is remarkable that more than 50% of western European and North American healthy individuals have circulating B-cells that carry this translocation.[10] In fact, the frequency of positive cells is between 0.1 and 100 per 1 million cells, and there are hints that these cells can persist for some time.[11–13] The t(14;18)-translocations in cells of healthy individuals are indistinguishable from those found in follicular lymphoma, and the strongly conserved breakpoints within the BCL2 gene and the immunoglobulin heavy chain locus make this aberration a prime target for highly sensitive DNA-PCR assays. Indeed, a number of different PCR assays have been established for the molecular monitoring of t(14;18)-positive lymphoma cells in the peripheral blood, lymph nodes and bone marrow of FL patients.[14,15] Moreover, with the introduction of quantitative real-time PCR, it has become possible to obtain reliable quantitative data, which in turn allows monitoring of the dynamics of the disease.[16] To this end, we previously described a highly reproducible standardized quantitative real-time PCR assay for the detection of the t(14;18)-MBR (major breakpoint region) translocation.[8,17]

In this study, we used this technique to assess the age-dependent prevalence and frequency of the t(14;18)-MBR-rearrangements in the peripheral blood of a large cohort of healthy individuals whose age ranged from 0 to 91 years old, with the aim of approaching the significance of these cells as clonal lymphoma precursors or indicators of lymphoma risk when comparing these results with numbers of circulating t(14;18)-positive cells in FL patients.

Material and methods

Peripheral blood samples and DNA isolation

A total of 715 peripheral blood samples were collected and analyzed as part of this study, with subjects ranging in age from newborn to 91 years old. Most of these samples (444/715) were taken during routine physicals of healthy individuals, after informed consent was obtained. To make it possible to analyze roughly equal numbers of samples within different

age groups, additional (271/715) samples were obtained from other sources. The regional SHIP-study (Study of Health in Pomerania)[18] provided 140 samples from individuals between 50 and 80 years of age. In addition, the Department of Pediatrics of the Ernst-Moritz-Arndt-University Greifswald supplied 36 cord blood samples, 48 peripheral blood samples from children less than 10 years of age and 47 peripheral blood samples from children and adolescents between 10 and 19 years of age. None of these individuals exhibited evidence of a lymphoproliferative disease, and all aspects of this study were approved by our institutional ethics committee.

Furthermore, a total of 327 peripheral blood samples were obtained from 108 consecutive follicular lymphoma patients with stage III/IV at initial presentation who were found to have circulating t(14;18)-MBR-positive cells by the quantitative real-time PCR method described below.

Peripheral blood mononuclear cells (PBMNCs) were isolated by density gradient centrifugation and stored at −20°C until needed. Standard techniques were used to prepare high molecular-weight DNA from these cells.[11,17]

Quantitative real-time PCR

Quantitative real-time PCR for the t(14;18)-MBR translocation was carried out in PBMNCs as described previously by Dälken *et al.*,[17] using the ABI PRISM 7700 Sequence Detection System (PE Applied Biosystems). Spectrophotometric measurements of DNA levels were used to obtain DNA from roughly 140,000 cells. PCR of wild-type K-ras was carried out as a reference test for amplifiable DNA and to determine more accurately the number of cells analyzed in each single assay. Both probes were labeled with the reporter dye FAM (6-carboxy-fluorescein) at the 5′-end and the quencher dye TAMRA (6-carboxy-tetramethylrhodamine) at the 3′-end. The PCR mixture contained each forward and reverse primer at a concentration of 400 nM, probe at a concentration of 200 nM, the standard TaqMan Universal PCR Master Mix (PE Applied Biosystems) and between 0.1 and 2.0 μg DNA. After a 2-min incubation at 50°C to allow for cleavage by Uracil-*N*-Glycosylase (UNG), AmpliTaq Gold was activated by incubation at 95°C for 10 min. Each PCR cycle consisted of 15 sec denaturation at 95°C, and 1 min of combined annealing/extension at 61°C. In the cases of samples with low copy numbers, the number of PCR cycles was increased from 50 (as previously described) to 55 to increase product yield for electrophoretical analysis. Standard curves were established for the t(14;18)-MBR translocation-specific PCR, as well as for the K-ras wild-type-specific PCR as described.[17]

The standard test procedure for the detection of the t(14;18)-translocations consisted of 5 PCR reactions with 1 μg DNA, plus 2 positive and 2 negative controls. As positive reference standards, we used the cloned DNA fragment of the t(14;18)-positive cell line Karpas 422 at a defined copy number (200 or 700 copies/assay). For the detection of wild-type K-ras, we performed 3 PCR reactions with 0.1 μg DNA, and again included 2 positive and 2 negative controls. As positive reference standards for the K-ras wild-type PCR, we used aliquots of cloned wild-type K-ras (1,000 copies/assay). For the test samples, if a negative t(14;18)-PCR result was obtained when fewer than 350,000 cells were represented in the 5 aliquots, additional DNA was analyzed (5 × 1 μg repeated) until at least 350,000 cells were analyzed.

The quantitative real-time PCR technique described here can detect the t(14;18)-translocation with a sensitivity of one copy in 1–2 μg of DNA, *i.e.*, one t(14;18)-positive cell in 140,000–280,000 normal cells.[17]

Electrophoresis of PCR products

The amplification products of all t(14;18)-positive samples were analyzed by agarose gel electrophoresis to identify samples with more than one t(14;18)-positive clone. PCR products obtained using this quantitative real-time PCR assay usually run as a single specific band upon electrophoretical analysis. Nevertheless, because at least 5 PCR assays with 1 μg DNA were carried out for each sample, it was possible to detect multiple positive clones.

Sequence analysis of BCL2/IgH products

Nucleotide sequence analysis was carried out on selected t(14;18)-positive amplification fragments when similar size fragments were detected. Although all sequenced fragments harbored the translocations typical of FL, no identical sequences were found. For sequence analysis, real-time PCR products had to be subjected to a second round of standard PCR using the elongated primers 5′-GTAAGCTTAC CTGAGGAGAC GGTGAC-3′ and 5′-CGATGGATCC TGGTGGTTTG ACCTTTAGAG A-3′. PCR products were purified and sequenced using the ABI PRISM Sequence Analyzer 310 (Applied Biosystems).

Statistical analysis

χ^2-Test, Fisher's exact test, and nonparametric tests (Kruskal–Wallis and Mann–Whitney) were used for analyzing differences between groups (Software: SAS; SAS Institute, Cary, NC, v.9.1). A p-value <0.05 was considered statistically significant. A linear regression model was used to analyze the relationship between age and prevalence of t(14;18)-translocations (software: GraphPad PRISM v.5.0, GraphPad Software Inc., La Jolla, CA; www.graphpad.com).

We calculated the density distribution based on the number of t(14;18)-positive cells divided by the number cells tested on our subgroup of t(14;18)-positive individuals. Assuming a binomial distribution of the frequencies of t(14;18)-positive cells, we also calculated the probability to correctly identify t(14;18)-positive individuals based on a given number of cells and the number of cells that have to be tested when a positive PCR result with a probability of 95% will be obtained. This calculation was done by using the software "INTERCOOLED STATA" version 8.0, StataCorp LP, College Station, TX, USA; (www.stata.com).

Results

Prevalence and frequency of t(14;18)-MBR translocation

DNA from 715 healthy individuals was tested for the presence of the t(14;18)-MBR translocation. The overall median number of cells tested per subject across age groups (taking into account all samples analyzed, whether positive or negative), was 644,000 cells (Table I). The mean prevalence of t(14;18)-MBR-positive cells in peripheral blood lymphocytes from healthy individuals was 45.7% (327/715). The frequency of circulating

t(14;18)-positive cells in PCR-positive individuals varies from 0.5 to 1079 per 1 million PBMNCs, whereas the mean frequency was found to be 19.84×10^{-6}. Only 4.3% (31/715) of individuals carried more than one t(14;18)-positive cell per 25,000 PBMNCS (or >40/1 million). Among these individuals with high numbers of t(14;18)-positive cells, we did not observe any further increase in frequency with age. The highest frequency of 1079 t(14;18) positive cells/1 million PBMNCS was observed in a 44-year-old individual. In 17.9% (128/715) of all individuals and 39.1% of all positive individuals, we found more than 1 t(14;18)-positive cell clone. Four individuals showed 5 and 1 individual even showed 6 different PCR amplification fragments on electrophoretical analysis (Table II).

Age-dependence of t(14;18)-MBR translocation

The individuals under study were divided into 9 groups according to age (newborns [umbilical cord blood samples]; 0–9 [median age: 2.6]; 10–19 [median age: 16.1]; 20–29 [median age: 25]; 30– 39 [median age: 36]; 40–49 [median age: 44]; 50–59 [median age: 54]; 60–69 [median age: 63]; 70–91 [median age: 74]). The prevalence of t(14;18)-positive cells was strongly correlated with age (Fig. 1; χ^2-test: $p < 0.0001$). A linear regression model ($y = 1.597x–8.274$; $R^2 = 0.9702$) could be successfully applied to the trend of increasing prevalence with age, rising by a rate of 1.6% per year between 5 years of age and continuing through to 44 years of age. The highest prevalence, of about 66%, was found within age group 40–49 years. No further increase in prevalence was observed in the large group of individuals greater than 50 years old.

Within the subgroup of all t(14;18)-positive individuals, the proportion of having more than one t(14;18)-positive cell clone was also clearly associated with age (Table II; χ^2-test: $p = 0.00351$). Multiple clones were found only in individuals greater than 20 years old. The proportion of multiple clones among positives ranged from 33.3% in age group 20–29 years to 48.9% age group 70–91 years.

The median frequencies of t(14;18)-positive cells among positive individuals was strongly associated with age (Fig. 2; Kruskal-Wallis Test: $p < 0.0001$). Applying Mann–Whitney test to groups older than 9 years showed significantly higher median frequencies of t(14;18)-positive cells in individuals greater than 40 years old in comparison to individuals less than 40 years old ($p < 0.0001$). Regarding individuals with high numbers of circulating t(14;18)-positive cells with more than one t(14;18)-positive cell in 25,000 PBMNCS (see Fig. 2 and Table I), all except one were over 40 years old (Fisher's exact test: $p < 0.0001$).

Probability of detection relative to number of cells tested

An explorative analysis to estimate the number of cells required to correctly identify t(14;18)-positive individuals was conducted. First, we analyzed the proportion of t(14;18)-positive individuals as a function of the number of cells tested (data not shown but provided in Supporting Information). In this analysis, there was no tendency toward higher prevalence for larger numbers of cells analyzed.

Actually, a typical translocation frequency is not known, because there is a broad range (about 1–1,000/10^6 PBMNC) in numbers of circulating t(14;18)-positive cells in healthy individuals. Therefore, we determined the average frequency of t(14;18) positive cells from

all individuals with a positive PCR-result in our sample. On the basis of this frequency, we calculated a binomial density distribution in regard to different numbers of cells analyzed. For the prevalence observed in our sample, 95% of t(14;18)-positive individuals with the average frequency of 19.8×10^{-6} t(14;18)-positive cells (or 1 t(14;18)-positive cell per 50,505 PBMNC) would be detected by analysis of 107,700 cells. Because a median number of 644,000 cells was tested in our cohort, we assume that the identification of t(14;18)-positive individuals in our cohort with ages between 10 and 91 years ($n = 630$) was complete, with an overall prevalence of 51.9%. On the ordinate of Figure 3, the probability to correctly identify t(14;18)-positive individuals is shown. The solid line in Figure 3 represents this situation. Furthermore, the probabilities to detect a positive result at various numbers of cells tested can be read off in this figure, because this approach was further used to estimate the probability of obtaining a positive PCR result when using QRT-PCR to test for the t(14;18)-MBR rearrangement at different situations, *i.e.*, when increasing amounts of DNA were used in assays on samples with different mean frequencies of t(14;18)-positive cells (Fig. 3, Table III). The probability to detect circulating t(14;18)-positive cells predominantly depends on the amount of DNA used for the assay, *e.g.*, 95% of t(14;18)-positive individuals with the average frequency of 10×10^{-6} t(14;18)-positive cells would be detected by analysis of 300,000 cells, whereas an average frequency of 4×10^{-6} requires a number of about 750,000 cells (Table III). But to a lesser extent this probability also depends on the age composition of the study population (data not shown).

In case of comparable PCR assays applied by different groups, this model can be used to compare the results on prevalence of t(14;18)-positive cell in healthy individuals between different studies when the DNA amount used for PCR analysis and age composition of the study population are known.

Translocation in healthy individuals *versus* FL patients at primary diagnosis

The number of circulating t(14;18)-MBR positive cells of the 327 t(14;18)-positive healthy individuals was significantly lower than those found in 108 consecutive patients with stage III/IV FL at first presentation with t(14;18)-positive cells detectable in their peripheral blood (Fig. 4). The median frequency of t(14;18)-positive cells in healthy individuals was 5.8/1 million PBMNCS (range 0.5–1079), whereas in FL patients the median number of circulating lymphoma cells was about 9200/1 million PBMNCS (range 7–1,000,000). Whenever possible, the t(14;18) PCR fragment amplified from PBMNCs of FL patients was validated by PCR analysis of DNA isolated from primary diagnostic tissue lymph-node biopsies. Although we note that there is overlap in the frequencies of t(14;18)-MBR-positive cells found in healthy individuals and FL patients who have not yet undergone therapy, our data suggest that a level of greater than 1 t(14;18)-positive cell in 1,000 PBMNCs is pathognomonic for patients with malignant lymphoma.

Discussion

In this study, which used a standardized, highly sensitive quantitative real-time PCR technique, we assessed the prevalence and frequency of the BCL2/IgH rearrangement—which is characteristic of the t(14;18)-MBR translocation that is associated with follicular

lymphoma (FL)—in 715 DNA samples from healthy individuals. The mean prevalence of circulating t(14;18)-positive cells in PBMNCs from all healthy individuals was found to be 46% (327/715). Moreover, 4.3% of the individuals tested showed a significantly elevated level of this translocation (defined by >40/10^6), in support of results from previous studies on smaller numbers of individuals.[10–12,19]

The t(14;18)-translocation is thought to develop early in B-cell development, during the recombination of the D- and J- genes of the immunoglobulin heavy chain locus within the bone marrow.[20] Newer studies support this hypothesis when clonotypic sequences of the t(14;18) translocation were determined in different B-cell subsets and found to be present at a lower level in antigen naïve (CD27-) cells.[21] Therefore, most of these translocations are likely to occur during childhood and early adulthood, when the immune system is most active. However, it may take years before these cells can be detected in the peripheral blood.

Our data suggest that circulating t(14;18)-positive cells cannot be easily detected by established PCR techniques until the age of 9. Strikingly, in spite of the fact that we tested 84 peripheral blood samples from children, including 36 umbilical cord blood samples, this genetic aberration was not seen in a child under the age of 10. Circulating t(14;18)-positive cells were first observed in children aged between 10 and 19 years old, with an average prevalence of about 19%. These results support the idea that t(14;18)-positive cells primarily reside in lymphoid tissues where the microenvironment possibly stimulates proliferation, clonal persistence and expansion of at least some positive clones.[7] The number of tissue samples studied for t(14;18)-positive cells is rather low compared to the large number of peripheral blood samples, especially in young children. Indeed, t(14;18)-positive cells have been detected in tonsils with benign follicular hyperplasia (BFH) (4 out of 8 children aged between 4 and 8 years)[22] and reactive lymphoid tissues of children (2 out of 8 children aged between 2 and 9) by PCR.[23] It is interesting to note that the distribution of BCL2/IgH-positive cells in children's tonsils with BFH was not uniform, but clustered within microscopically dissected follicles.[22] In addition, 2 different translocations were observed in one tonsil.

It is notable that although some previous studies have shown an increasing prevalence of BCL2/IgH rearrangement with age,[24,25] others have failed to do so.[11,19,26–28] The data presented here clearly show an increasing prevalence of t(14;18)-positive cells up through the age of about 50 (Fig. 1). These findings are consistent with those of 3 previous studies that found t(14;18)-positive cells in only 4/35 (11.5%) of children between 0 and 19 years of age.[24,25,28] Another study by Yasukawa *et al.*,[29] which involved Japanese subjects, found t(14;18)-positive cells in only 1/49 (2%) of children (0–19 years old) compared to an average of about 20% in adults, which is in keeping with a lower incidence of FL in the Japanese compared to the Caucasian population. Taken together, these results are consistent with the relatively low incidence of FL in humans until the age of 40.

We found significantly higher median frequencies of t(14;18)-positive cells in individuals over 60 years old (Fig. 2). Except in one case, we detected high levels of t(14;18)-positive cells (more than 1 in 25,000 PBMNCs) only in individuals who were over 40 years old. On the other hand, the absence of samples with more than 1 t(14;18)-positive cell in 1,000

PBMNC, among very old people (>60 years old), together with the almost constant presence of t(14;18)-positive cells in individuals over 50 years old, implies that the growth of these cells continues to be regulated, either by a cellular mechanism or by the immune system, *e.g.*, the idiotype anti-idiotype network.

With regard to mechanisms of repression, it has been shown that t(14;18)-positive cells can undergo further maturation (immunoglobulin class-switch recombination), *i.e.*, that they are not naïve anymore but capable of responding to a stimulus by an exogenous antigen.[7] On the other hand, in an analysis of individuals from whom matched samples for a 3 year follow-up were available (mean age: 42.5 years), Roulland *et al.*[13] found that 15/26 (57.6%) had significantly increasing numbers of t(14;18) positive cells. However, it remains unclear whether the number of these cells is actually maintained or these cells are released from a certain pool by antigenic stimulation, with the latter resulting in a dynamic accumulation of t(14;18)-positive cells to the levels that are detected in FL patients at first presentation.

Whether individuals with high numbers of t(14;18)-positive cells (Fig. 2) or those with multiple t(14;18)-positive clones (Table II) are at an increased risk of lymphoid malignancies still remains unanswered. Indeed, this suggests that additional genetic events in one of these cells, or perhaps a loss of control by the immune system, may be involved in the pathway to malignant lymphoma. On the other hand, the circulating t(14;18)-positive cells in healthy individuals with about 100 t(14;18) positive cells/1 million PBMNC is only 100-fold, or about 7–8 doublings, different from those of FL patients with a median number of about 9200 t(14;18)-positive cells per 1 million PBMNCs (Fig. 4). A definite investigation would require a sufficiently large, population based, and prospective longitudinal study.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

T. Berg was supported by a grant from the “Alfried-Krupp-Graduiertenkolleg Tumorbiologie” provided by the “Alfried Krupp Wissenschaftskolleg Greifswald”. SHIP is part of the Community Medicine Research net (CMR) of the University of Greifswald, Germany, which is funded by the Federal Ministry of Education and Research (grant no. ZZ9603) and the Ministry of Cultural Affairs as well as the Social Ministry of the Federal State of Mecklenburg-West Pomerania (http://www.community-medicine.de).

References

1. Cleary ML, Smith SD, Sklar J. Cloning and structural analysis of cDNAs for bcl-2 and a hybrid bcl-2/immunoglobulin transcript resulting from the t(14;18) translocation. Cell. 1986; 47:19–28. [PubMed: 2875799]
2. Fukuhara S, Rowley JD, Variakojis D, Golomb HM. Chromosome abnormalities in poorly differentiated lymphocytic lymphoma. Cancer Res. 1979; 39:3119–3128. [PubMed: 582296]
3. Yunis JJ, Oken MM, Kaplan ME, Ensrud KM, Howe RR, Theologides A. Distinctive chromosomal abnormalities in histologic subtypes of non-Hodgkin’s lymphoma. N Engl J Med. 1982; 307:1231–1236. [PubMed: 7133054]

4. Cong P, Raffeld M, Teruya-Feldstein J, Sorbara L, Pittaluga S, Jaffe ES. In situ localization of follicular lymphoma: description and analysis by laser capture microdissection. Blood. 2002; 99:3376–3382. [PubMed: 11964306]
5. Egle A, Harris AW, Bath ML, O'Reilly L, Cory S. VavP-Bcl2 transgenic mice develop follicular lymphoma preceded by germinal center hyperplasia. Blood. 2004; 103:2276–2283. [PubMed: 14630790]
6. McDonnell TJ, Deane N, Platt FM, Nunez G, Jaeger U, McKearn JP, Korsmeyer SJ. bcl-2-immunoglobulin transgenic mice demonstrate extended B cell survival and follicular lymphoproliferation. Cell. 1989; 57:79–88. [PubMed: 2649247]
7. Roulland S, Navarro JM, Grenot P, Milili M, Agopian J, Montpellier B, Gauduchon P, Lebailly P, Schiff C, Nadel B. Follicular lymphomalike B cells in healthy individuals: a novel intermediate step in early lymphomagenesis. J Exp Med. 2006; 203:2425–2431. [PubMed: 17043145]
8. Dölken G. Detection of minimal residual disease. Adv Cancer Res. 2001; 82:133–185. [PubMed: 11447762]
9. Bende RJ, Smit LA, van Noesel CJ. Molecular pathways in follicular lymphoma. Leukemia. 2007; 21:18–29. [PubMed: 17039231]
10. Schüler F, Hirt C, Dölken G. Chromosomal translocation t(14;18) in healthy individuals. Semin Cancer Biol. 2003; 13:203–209. [PubMed: 12959351]
11. Dölken G, Illerhaus G, Hirt C, Mertelsmann R. BCL-2/JH rearrangements in circulating B cells of healthy blood donors and patients with nonmalignant diseases. J Clin Oncol. 1996; 14:1333–1344. [PubMed: 8648392]
12. Limpens J, Stad R, Vos C, de Vlaam C, de Jong D, van Ommen GJ, Schuuring E, Kluin PM. Lymphoma-associated translocation t(14;18) in blood B cells of normal individuals. Blood. 1995; 85:2528–2536. [PubMed: 7727781]
13. Roulland S, Lebailly P, Lecluse Y, Heutte N, Nadel B, Gauduchon P. Long-term clonal persistence and evolution of t(14;18)-bearing B cells in healthy individuals. Leukemia. 2006; 20:158–162. [PubMed: 16307019]
14. Schüler F, Dölken G. Detection and monitoring of minimal residual disease by quantitative real-time PCR. Clin Chim Acta. 2006; 363:147–156. [PubMed: 16154122]
15. Johnson PW, Swinbank K, MacLennan S, Colomer D, Debuire B, Diss T, Gabert J, Gupta RK, Haynes A, Kneba M, Lee MS, Macintyre E, et al. Variability of polymerase chain reaction detection of the bcl-2-IgH translocation in an international multicentre study. Ann Oncol. 1999; 10:1349–1354. [PubMed: 10631464]
16. Darby AJ, Lanham S, Soubeyran P, Johnson PW. Variability of quantitative polymerase chain reaction detection of the bcl-2-IgH translocation in an international multicenter study. Haematologica. 2005; 90:1706–1707. [PubMed: 16330451]
17. Dölken L, Schüler F, Dölken G. Quantitative detection of t(14;18)-positive cells by real-time quantitative PCR using fluorogenic probes. Biotechniques. 1998; 25:1058–1064. [PubMed: 9863062]
18. John U, Greiner B, Hensel E, Ludemann J, Piek M, Sauer S, Adam C, Born G, Alte D, Greiser E, Haertel U, Hense HW, et al. Study of health in pomerania (SHIP): a health examination survey in an east German region: objectives and design. Soz Praventivmed. 2001; 46:186–194. [PubMed: 11565448]
19. Summers KE, Goff LK, Wilson AG, Gupta RK, Lister TA, Fitzgibbon J. Frequency of the Bcl-2/IgH rearrangement in normal individuals: implications for the monitoring of disease in patients with follicular lymphoma. J Clin Oncol. 2001; 19:420–424. [PubMed: 11208834]
20. Tsujimoto Y, Finger LR, Yunis J, Nowell PC, Croce CM. Cloning of the chromosome breakpoint of neoplastic B cells with the t(14;18) chromosome translocation. Science. 1984; 226:1097–1099. [PubMed: 6093263]
21. Hirt C, Dölken G, Janz S, Rabkin CS. Distribution of t(14;18)-positive, putative lymphoma precursor cells among B-cell subsets in healthy individuals. Br J Haematol. 2007; 138:349–353. [PubMed: 17614821]

22. Limpens J, de Jong D, van Krieken JH, Price CG, Young BD, van Ommen GJ, Kluin PM. Bcl-2/JH rearrangements in benign lymphoid tissues with follicular hyperplasia. Oncogene. 1991; 6:2271–2276. [PubMed: 1766674]

23. Aster JC, Kobayashi Y, Shiota M, Mori S, Sklar J. Detection of the t(14;18) at similar frequencies in hyperplastic lymphoid tissues from American and Japanese patients. Am J Pathol. 1992; 141:291–299. [PubMed: 1497088]

24. Ji W, Qu GZ, Ye P, Zhang XY, Halabi S, Ehrlich M. Frequent detection of bcl-2/JH translocations in human blood and organ samples by a quantitative polymerase chain reaction assay. Cancer Res. 1995; 55:2876–2882. [PubMed: 7796416]

25. Liu Y, Hernandez AM, Shibata D, Cortopassi GA. BCL2 translocation frequency rises with age in humans. Proc Natl Acad Sci USA. 1994; 91:8910–8914. [PubMed: 8090743]

26. Bell DA, Liu Y, Cortopassi GA. Occurrence of bcl-2 oncogene translocation with increased frequency in the peripheral blood of heavy smokers. J Natl Cancer Inst. 1995; 87:223–224. [PubMed: 7707410]

27. Fuscoe JC, Setzer RW, Collard DD, Moore MM. Quantification of t(14;18) in the lymphocytes of healthy adult humans as a possible biomarker for environmental exposures to carcinogens. Carcinogenesis. 1996; 17:1013–1020. [PubMed: 8640906]

28. Rauzy O, Galoin S, Chale JJ, Adoue D, Albarede JL, Delsol G, al Saati T. Detection of t(14;18) carrying cells in bone marrow and peripheral blood from patients affected by non-lymphoid diseases. Mol Pathol. 1998; 51:333–338. [PubMed: 10193514]

29. Yasukawa M, Bando S, Dolken G, Sada E, Yakushijin Y, Fujita S, Makino H. Low frequency of BCL-2/J(H) translocation in peripheral blood lymphocytes of healthy Japanese individuals. Blood. 2001; 98:486–488. [PubMed: 11435322]

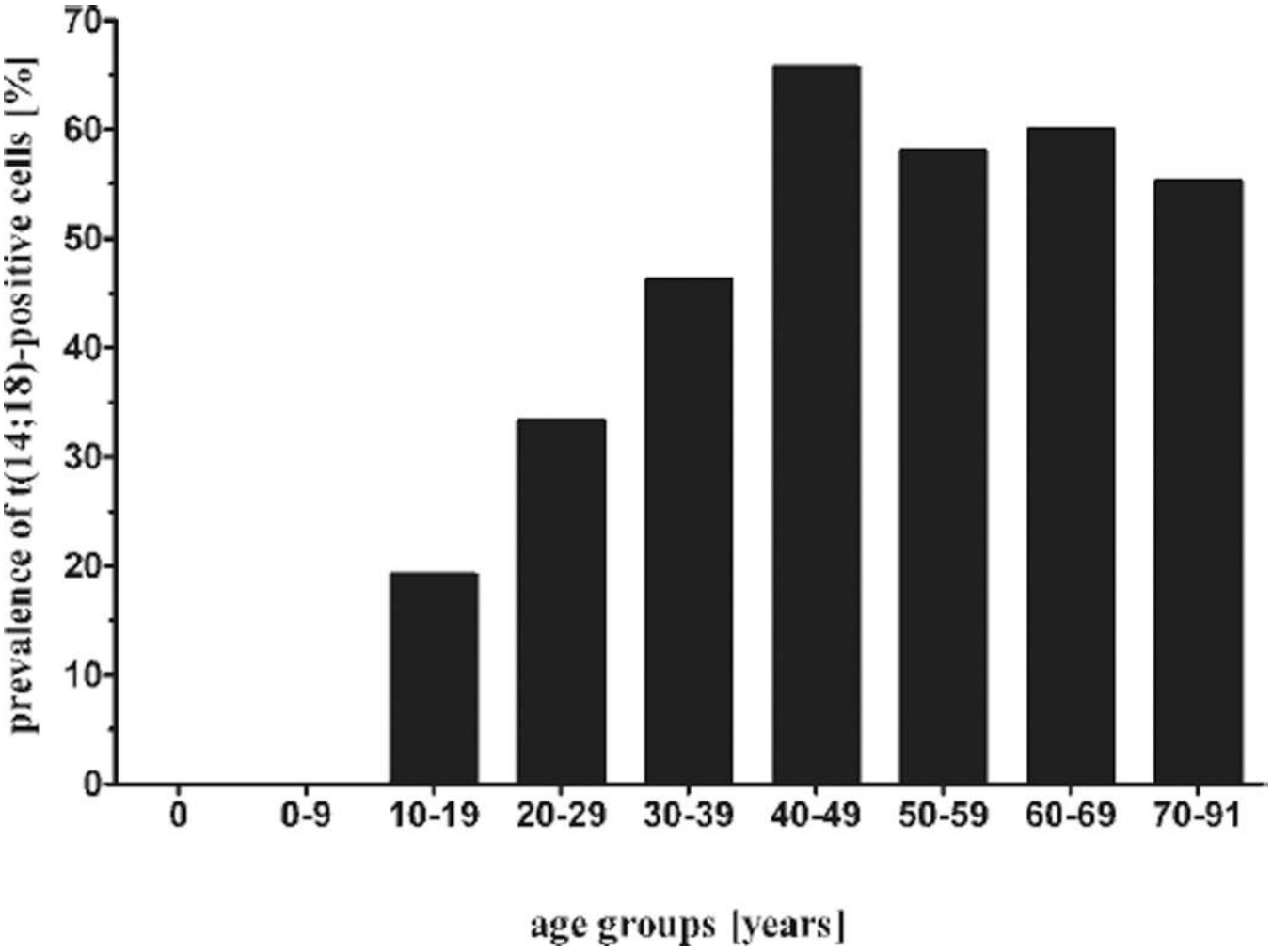

Figure 1.

Prevalence of circulating t(14;18)-MBR-positive cells in various age groups detected by quantitative real-time PCR (for further details, see Table I).

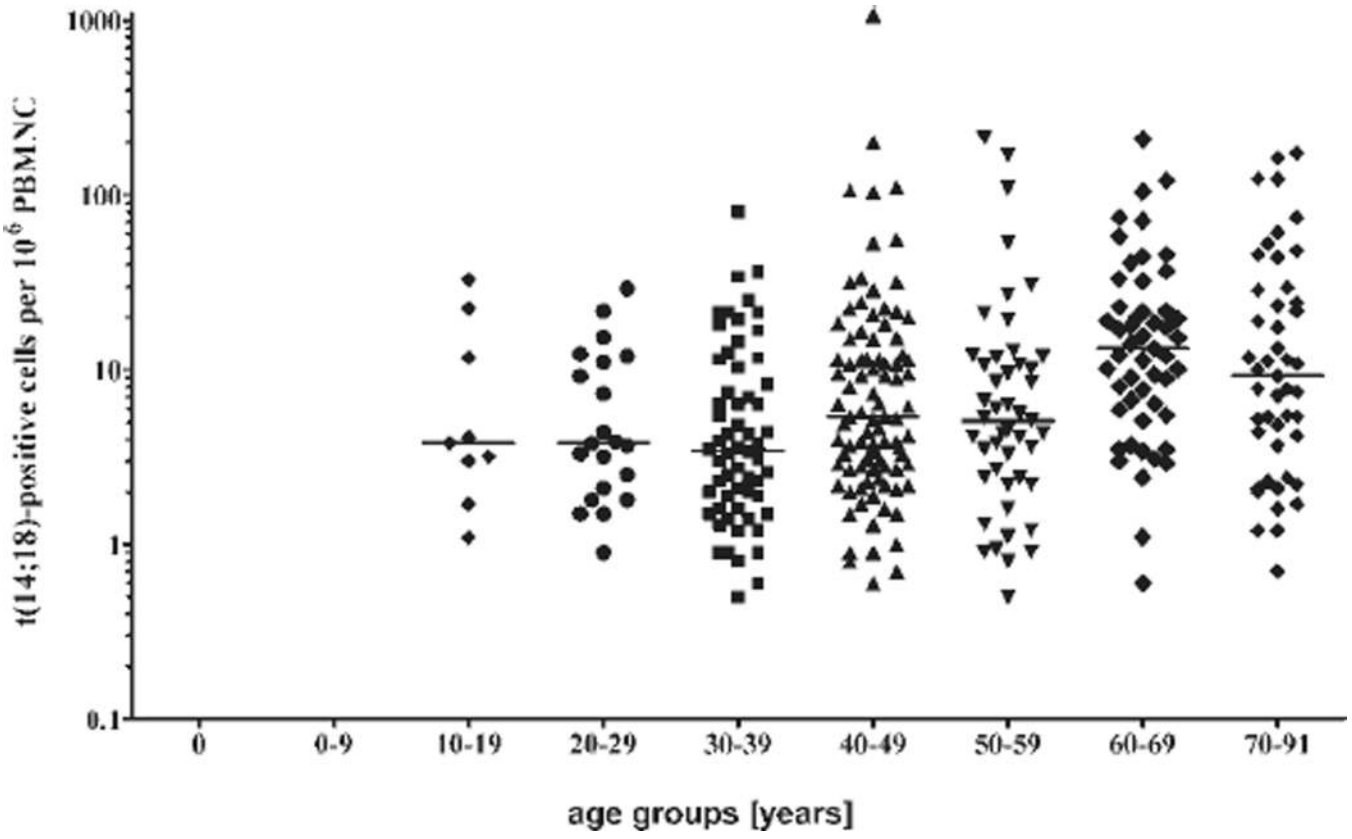

Figure 2.

Frequency of circulating t(14;18)-MBR-positive cells in various age groups as determined by quantitative real-time PCR. The horizontal lines identify the median number of circulating t(14;18)-positive cells within the subgroup all t(14;18)-positive individuals (for further details, see Table I).

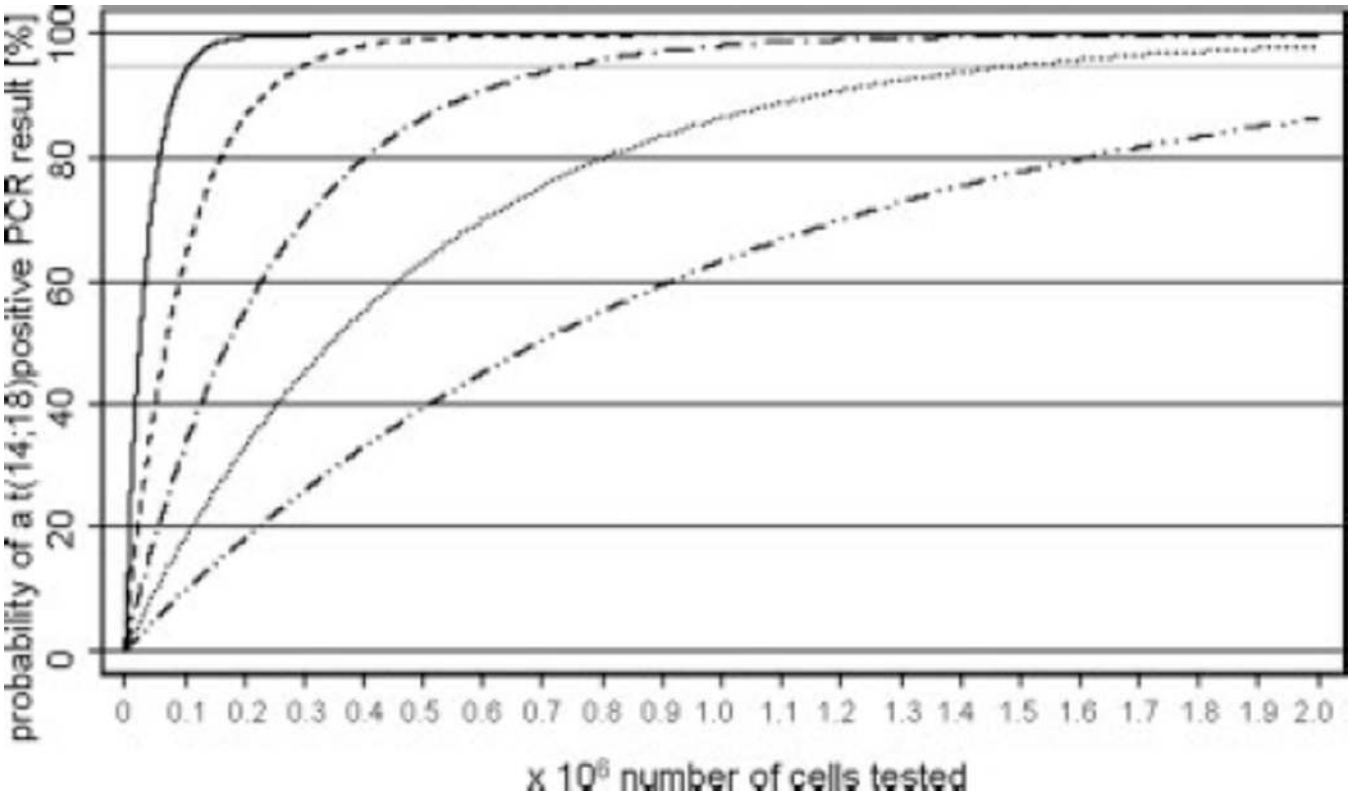

Figure 3.

The density distribution shows the average probability to correctly identify a t(14;18)-positive individual in our sample (mean frequency 19.8×10^{-6}) depending on the number of cells analyzed (——). For example, when 151,200 cells per individual were analyzed we would have detected 95% of the prevalent cases. This method was further applied to calculate the probability of obtaining a t(14;18)-positive PCR result in truly positive individuals at various sensitivity levels (assumed mean frequencies): 10×10^{-6} (- - - - - - -); 4×10^{-6} (— · — · — · -); 2×10^{-6} (.....); 1×10^{-6} (— · · — · · —). This graph could be used to compare different studies with different sensitivities. The lower the number of cells tested is and the lower the mean frequency the lower is the probability to obtain a positive PCR result, *e.g.*, when an average number of 500,000 cells were tested the overall probability would only be about 65% to detect an individual with a frequency of 2×10^{-6} circulating t(14;18) positive cells.

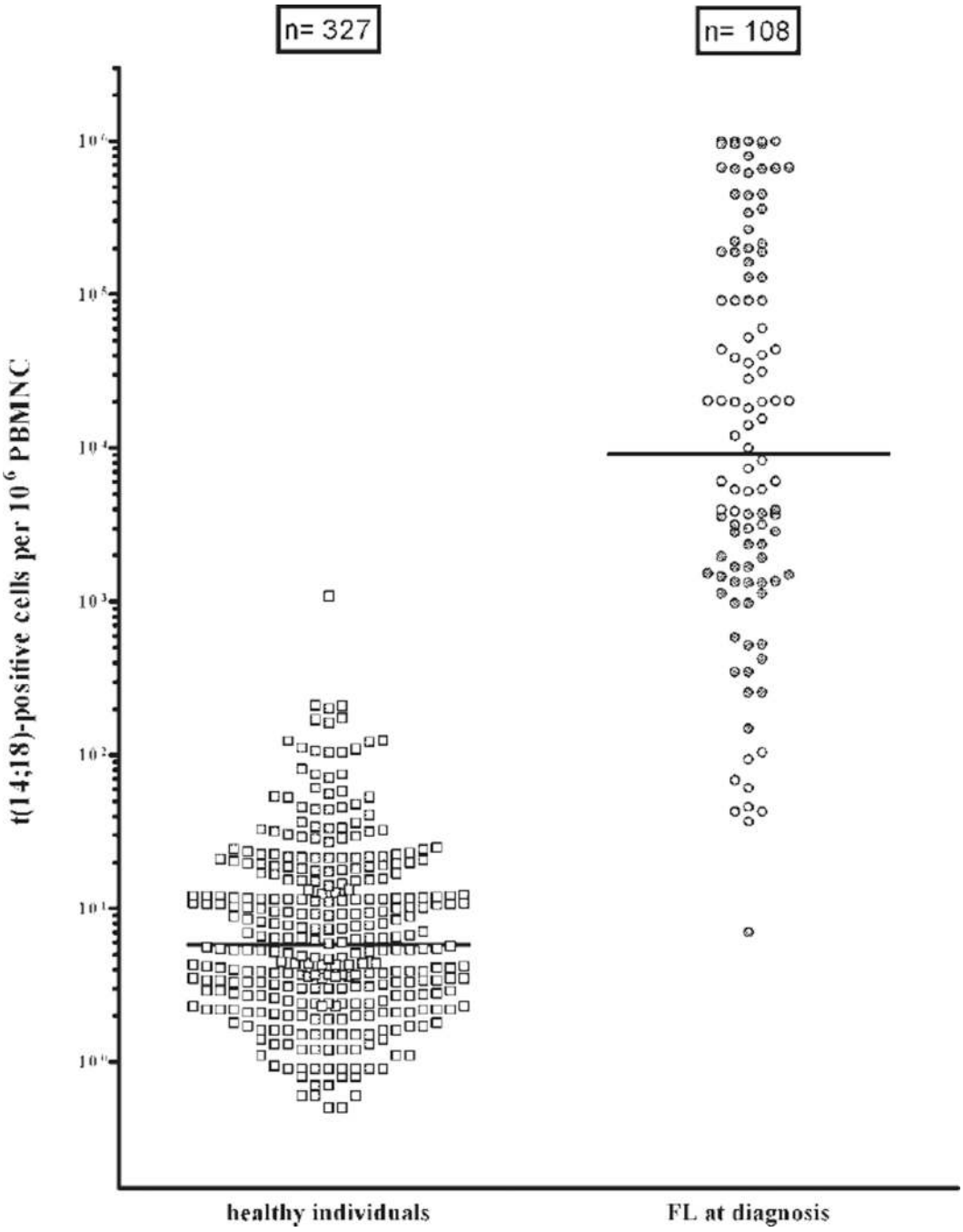

Figure 4.

Comparison of the number of circulating t(14;18)-positive cells in 327 healthy individuals with those found in 108 FL patients at first presentation. The median number of circulating t(14;18)-positive cells indicated by the solid line is 5.8/1 million PBMNC in healthy individuals compared to 9200/1 million in FL patients. In case of patients with less than 1 t(14;18)-positive cell in $10^{3,4}$ PBMNCs at primary diagnosis, quantitative PCR was

performed on lymph-node biopsies to identify the t(14;18) amplification fragment of the primary lymphoma cell clone.

TABLE I

RESULTS OF QUANTITATIVE REAL-TIME PCR ANALYSIS OF t(14;18)-MBR TRANSLOCATION IN HEALTHY INDIVIDUALS (n = 715) AGED 0–91 YEARS WITH RESPECT TO PREVALENCE AND FREQUENCY OF CIRCULATING t(14;18)-POSITIVE CELLS

Age (years)	*n*	Median age	Prevalence of t(14;18)-positive individuals (%)	Median frequency of t(14;18)-positive cells within the subgroups of all t(14;18)-positive individuals [10^{-6}]	Mean frequency of t(14;18)-positive cells within the subgroups of all t(14;18)-positive individuals [10^{-6}]	Median number of cells tested	Healthy individuals with > 40 t(14;18)-positive cells/10^6 PBMNC (%)
0 (cord blood)	36	0	0/36 (0)			993,000	0 (0)
0–9	48	3	0/48 (0)			493,000	0 (0)
10–19	47	16	9/47 (19)	3.8	9.3	437,000	0 (0)
20–29	63	25	21/63 (33)	3.8	7.3	631,000	0 (0)
30–39	130	36	60/130 (46)	3.5	8.2	769,000	1 (0.8)
40–49	140	44	92/140 (66)	5.4	26.1	796,000	7 (5)
50–59	81	54	47/81 (58)	5.1	17.9	817,000	4 (5)
60–69	85	63	51/85 (60)	13.2	25.1	742,000	9 (11)
70–91	85	74	47/85 (55)	9.2	26.4	681,000	10 (12)
All healthy individuals	715		327/715 (46)	5.8	19.8	644,000	31 (4)

TABLE II

THE PRESENCE AND NUMBER OF MULTIPLE t(14;18)-POSITIVE CLONES DETECTED IN PERIPHERAL BLOOD MONONUCLEAR CELLS OF HEALTHY INDIVIDUALS (n = 715) ACCORDING TO AGE

Number of different t(14;18)-positive clones	Age group							
	0–19	10–19	20–29	30–39	40–49	50–59	60–69	70–91
Only 1	0	9	14	35	55	35	27	24
2	0	0	7	16	21	7	12	15
3	0	0	0	4	13	3	9	7
4	0	0	0	2	2	2	3	0
5	0	0	0	3	1	0	0	0
6	0	0	0	0	0	0	0	1

TABLE III

THE NUMBER OF PBMNC THAT HAVE TO BE TESTED WHEN A POSITIVE T(14;18) PCR RESULT COULD BE EXPECTED WITH A PROBABILITY OF 95% AT DIFFERENT SENSITIVITY LEVELS

Mean frequency of t(14;18)-positive cells [10^{-6}]	Number of PBMNC
19.8	151,200
10	299,500
4	748,800
2	1,497,800
1	2,995,800

This model is based on the binomial distribution of circulating t(14;18)-positive cells among all circulating cells.