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Frequency of pseudo-Gaucher cells in diagnostic bone marrow biopsies from patients with Ph-positive chronic myeloid leukaemia

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Abstract Pseudo-Gaucher cells (PGC) are a characteristic finding in Ph-positive CML, and prolongation of survival was observed when PGC were detected within the bone marrow. However, the conspicuous variation in the reported frequencies indicates the necessity for analysis of their natural occurrence in the bone marrow from untreated CML patients. A total of 833 diagnostic bone marrow biopsies from patients with Ph-positive CML were examined for PGC by 7 observers. Proof of PGC was based on systematic examination of Giemsa-stained slides with and without polarization at high magnification. Birefringence within the cytoplasm turned out to be highly specific for PGC. The risk of overlooking PGC was at least 80% when the number of these storing histiocytes was 70 per slide or less, and at least 50% when the total amount per slide was ≤ 250. This high risk of failure explained the disagreement among the authors. An intensive investigation by at least two observers is mandatory if results are to be evaluated in research. Under the conditions used in this study, the natural frequency of PGC within the bone marrow from untreated patients with a Ph-positive CML is much higher than assumed to date, amounting to about 70%. On the basis of these findings, the prognostic importance of PGC in CML must be evaluated critically.

Key words Storing histiocytes · Pseudo-Gaucher cells · Chronic myeloid leukaemia · Observer disagreement · Bone marrow biopsies

Introduction

Prolonged survival times of patients with chronic myeloid leukaemia (CML) have been reported when storing histiocytes, the Pseudo-Gaucher cells (PGC) have been detected in the bone marrow, either from aspirates or from biopsies [15, 18, 20–22]. These histiocytes have been included in a risk score subdividing the patients with Ph-positive CML into groups with different survival times according to the demonstration of PGC [22]. The frequency of occurrence of PGC reported, however, varies conspicuously between authors (Table 1).

Table 1 Reported frequencies of pseudo-Gaucher cells (PGC) in chronic myeloid leukaemia (CML): the percentages varied from less than 10% to more than 50%. If the authors had distinguished between PGC and other storing histiocytes, especially the sea-blue histiocytes or overlapping types, only the reported frequencies of PGC are stated (*BM* bone marrow)

	Reference	PGC-positive	%
BM aspirates	[9]	3/60	5
	[16]	9/60	15
	[1]	11/64	17
	[3]	11/49	22
	[17]	5/18	28
	[15]	25/69	36
BM biopsies	[23]	11/55	20
	[18–22]	9/30–47/130	36
	[8]	111/264	42
	[5]	168/309	54

Comparable differences in detection rates were recorded within our own laboratory when individual evaluations made in the same material by several observers were compared. Thus, an analysis was performed of the factors considered to influence the determination of PGC, because these differences in distribution had not been treated in the literature.

The determination of prognostic factors from biopsy and laboratory data is of considerable interest, and a critical analysis of the natural distribution of PGC in untreated patients is indicated. Thus, the occurrence of these histiocytes was determined in the diagnostic bone marrow biopsies (BMB) from 833 untreated patients with Ph-positive CML who were listed in the Bone Marrow Registry, Hannover, Germany or in the German CML Study I [12, 13], and the results received from a total of seven examiners were compared.

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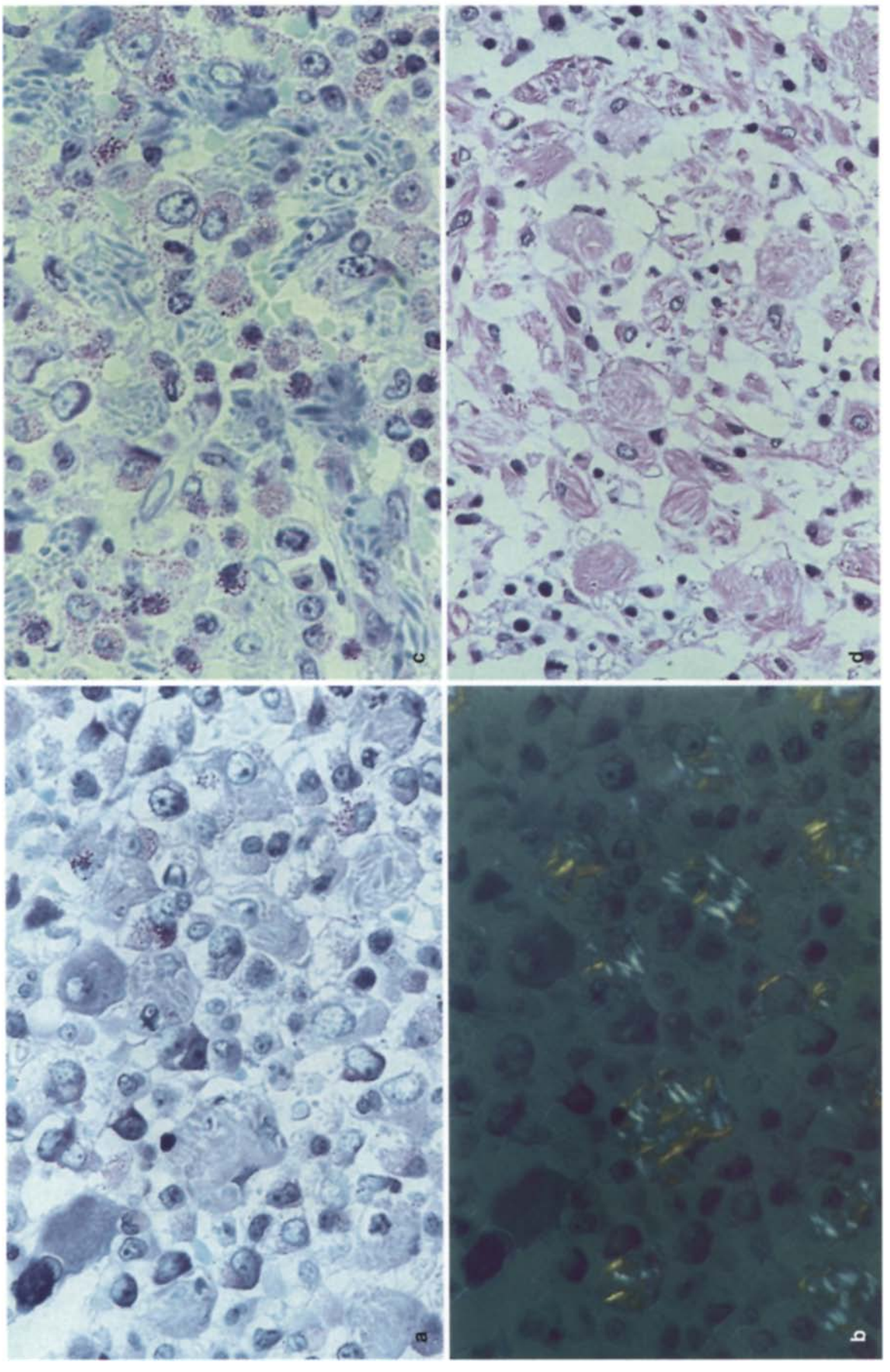
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Patients and methods

The diagnostic bone marrow biopsies (BMB) taken from a total of 833 patients with Ph-positive CML before the start of therapy were examined for Pseudo-Gaucher cells (PGC) independently by a total of seven observers: 739 BMB were embedded in methylmethacrylate (MMA) [4, 10] and 94 BMB were embedded in paraffin wax [24]. Five hundred and thirty-two patients examined by observer G were registered in the Bone Marrow Registry, Hannover, and 301 patients examined by observers A–F were recruited into the German CML Study I [12, 13]. In addition, bone marrow aspirates of good quality, which were available from 21 untreated patients, were examined independently using a microscope of high quality, the observers being unaware of the results from the 21 BMB taken simultaneously.

In a first approach, 76 BMB from the German CML Study I [12, 13] were evaluated in order to check the rate of observer disagreement, each observer using his own microscope. In a second approach, one high-quality microscope was used; at least two systematic thorough examinations were performed from each bone marrow slide at high magnification (500×) with and without polarization, and a more rigid definition of "positive" cases was applied: (1) Only storing histiocytes almost indistinguishable from Gaucher cells by light microscopy (Giemsa staining, PAS) were considered to be PGC; (2) at least four definitive PGC had to be detected within a slide, otherwise a slide was defined as "negative", and (3) polarization of Giemsa-stained slides was used as additional confirmation, because birefringence within cytoplasm is a characteristic morphological feature of PGC [11, 17].

The results from examiners A, B and C were systematically checked for false results, and each had to justify his diagnosis in a critical microscope discussion. The total count of storing histiocytes per slide was performed by a systematic thorough examination at high magnification (350–500×) with and without polarization. The numerical densities of PGC were evaluated by a new morphometric method [6] examining the total marrow area of a slide (mean: 22.1 ± 14.2 mm^2) excluding bone area and artifacts. The 72 largest PGC-positive BMB were also examined for the inhomogeneity of PGC distribution within a slide by estimating the coefficient of variation of the numerical densities between 25 different marrow areas of equal size.

Statistical analyses were carried out with respect to overlooked and detected PGC-positive cases, the tests used being the Mann–Whitney U-test, the Kruskal–Wallis test, and Fisher's exact test if $n < 80$, and the χ^2-test if $n \geq 80$ (with Yates' correction if $n < 200$). The risk of overlooking PGC was estimated assuming that the PGC would have been detected in the case of a larger total count, a higher numerical density or a lower inhomogeneity of distribution within the slide applying the product limit estimate.

Results

Sensitivity and specificity of the method applied in order to prove the PGC

The cytoplasm of Gaucher cells and of PGC showed a fibrillar, silver foil or greaseproof-like cytoplasm with a pale grey-blue colouring in Giemsa staining (Fig. 1) and a strong PAS positivity. The birefringence of the cytoplasm in polarization was a characteristic feature of PGC, especially in Giemsa-stained slides (Fig. 1): more than 95% of a total of more than 100 000 evaluated Gaucher-like histiocytes (Giemsa staining, PAS positivity) showed birefringence in polarization, and 98.5% of the BMB with Gaucher-like histiocytes showed birefringence in the cytoplasm from at least 4 cells (Table 2). A biopsy was considered PGC-positive if at least 4 typical PGC were detected. The birefringence of the cytoplasm could also be detected within unstained histological slides and in the PGC-positive bone marrow aspirates. In contrast, none of the more than 4000 Gaucher cells eval-

◀ **Fig. 1a–d** Pseudo-Gaucher (PGC) cells and their differential diagnoses. **a** PGC (Giemsa, ×1250) resemble Gaucher cells (**d**, PAS reaction; 20-year-old male patient with Gaucher's disease; ×1000). Their Gaucher-like pale grey-blue cytoplasm (**a**) shows a fibrillar birefringence in polarization (**b**, Giemsa, ×1250). The birefringence is not detected in the cytoplasm from sea-blue histiocytes or histiocytes with green crystals (**c**, ×1250)

Table 2 Specificity and sensitivity of the method applied to prove PGC in BMB (panel results from 4 examiners, Giemsa staining): When a high-quality microscope was used, only 3/205 cases with Gaucher-like histiocytes showed no birefringence of cytoplasm in polarization. Only 1/203 cases positive for polarization did not show any typical PGC. In 29 biopsies with PGC, a significant additional proportion of histiocytes with overlapping features, i.e. partly Gaucher-like cytoplasm with sea-blue granules, a large amount of cell debris or few green crystals, were detected. Some of these histiocytes also showed birefringence

Evaluated BMB with:	Detected without polarization	Birefringence	%
Pseudo-Gaucher cells (CML)	205	202	98.5
Overlapping types (CML)	30	30	100
With PGC	29	29	
Without PGC		1	1
Controls			
Normal histiocytes	300	None	0
Histiocytes with cell debris (CML)	173	None	0
Histiocytes with green crystals (CML)	66	None	0
Sea-blue histiocytes (CML)	117	None	0
Gaucher cells (Gaucher's disease)	7	None	0

Table 3 Frequency of PGC among untreated CML patients: comparison of the individual results from seven examiners: with a high quality microscope which was also used by examiners E and G, no differences were detected among the individual results of the observers considering untreated patients from the German CML Study I and untreated patients recruited for the Bone Marrow Registry, Hannover. However, the discussion of dissent cases and a revision of the panel results disclosed a high percentage of positive cases that had been overlooked; 8/301 cases (2.7%) remained controversial

Experiment	Examined by	Ratio	%
1. First approach (German CML Study I)	Observer D	32/76	(42%)
	Observer E	44/76	(58%)
	Observer F	38/76	(50%)
2. Second approach (German CML Study I)	Observer A	154/301	(51%)
	Observer B	155/301	(52%)
	Observer C	159/301	(53%)
3. BM Registry Hannover	Observer G	264/532	(50%)
4. After panel discussion (from experiment 2)	Observers A, B, C	172/289	(60%)
5. Revision of panel diagnosis (from experiment 4)		202/293	(69%)

Table 4 Observer agreement, reproduction of results and individual error rate: the rate of observer agreement was significantly improved by use of the same high quality microscope throughout (second approach). Each observer was able to reproduce 95% of his results 6 months later without reference to his first results. The individual error rates were ≈ 20%, mainly by falsely negative results. Cases judged as positive which could not be confirmed in a critical microscope discussion by the other examiner(s) were exceptional (≈1%)

Inter-observer agreement

Comparison of 3 observers	Equal		Dissent		Significance
	Ratio	%	Ratio	%	
D, E, F	43/76	57%	33/76	43%	$P<0.00005$
A, B, C	248/301	82%	53/301	18%	

Reproduction of results after 6 months

Observer	Equal		Dissent: initial		Significance
	Ratio	%	Positive	Negative	
A	40/42	95%	0	2	$P>0.05$
B	54/57	95%	0	3	
C	53/55	96%	1	1	

Individual error rate

Observer	Correct		False		Significance
	Ratio	%	Positive	Negative	
A	245/301	81.4%	4	52	$P>0.05$
B	248/301	82.4%	3	50	
C	254/301	84.4%	2	45	

Table 5 Dissent cases: a high ratio of PGC-positive BMB was detected among dissent cases by critical microscope discussion. Even on review of the panel diagnosis, 30 further PGC-positive BMB were detected, 26 of which had been overlooked by each of the panel members; 8/301 cases (2.7%) remained controversial. A review of the panel diagnosis by two further experienced hematopathologists in the 20 cases initially judged as "negative" by at least two panel members confirmed the "positive" panel diagnosis

Microscope discussion of dissent cases

Before discussion	*n*	Panel diagnosis after microscope discussion			
		Positive	Negative		Frequency of PGC
Consent cases	248	131 (53%)	117 (47%)	$P<0.00005$	172/289 (60%)
Dissent cases	53	41 (77%)			?/12

Revision of diagnosis

Panel diagnosis	*n*	Revision of panel diagnosis		
		Positive	Negative	Frequency of PGC
Positive	20	20 (100%)	0 (0%)	202/293 (69%)
Negative	117	26 (22%)	91 (78%)	
Dissent	12	4 (33%)		?/8

Table 6 Comparison of paraffin and plastic embedding: the results from three observers (A, B, C) were compared. Neither the initial rate of observer disagreement nor the final results with respect to the frequency of PGC-positive cases were significantly influenced by the method of embedding

Embedding	Observer disagreement				Frequency of PGC[a]	
	Before discussion		Remaining controversial		Ratio	%
	Ratio	%	Ratio	%		
Methyl-methacrylate	38/207	18.4	4/207	1.9	143/203	70
Paraffin	15/94	16.0	4/94	4.3	59/90	66
Significance	$P>0.1$				$P>0.1$	

[a] Final result after revision of panel results

uated from the seven patients with Gaucher's disease showed any birefringence of the cytoplasm (Table 2).

The sensitivity of the birefringence depended heavily on the quality of the microscope used: only 40/84 PGC-positive cases showed birefringence within the cytoplasm in a minority of their Gaucher-like histiocytes when applying microscope I, which had initially been used by observer B, whereas more than 95% of the PGC within these 84 slides were birefringent with microscope II ($P<0.00005$), which was finally used by examiners A,

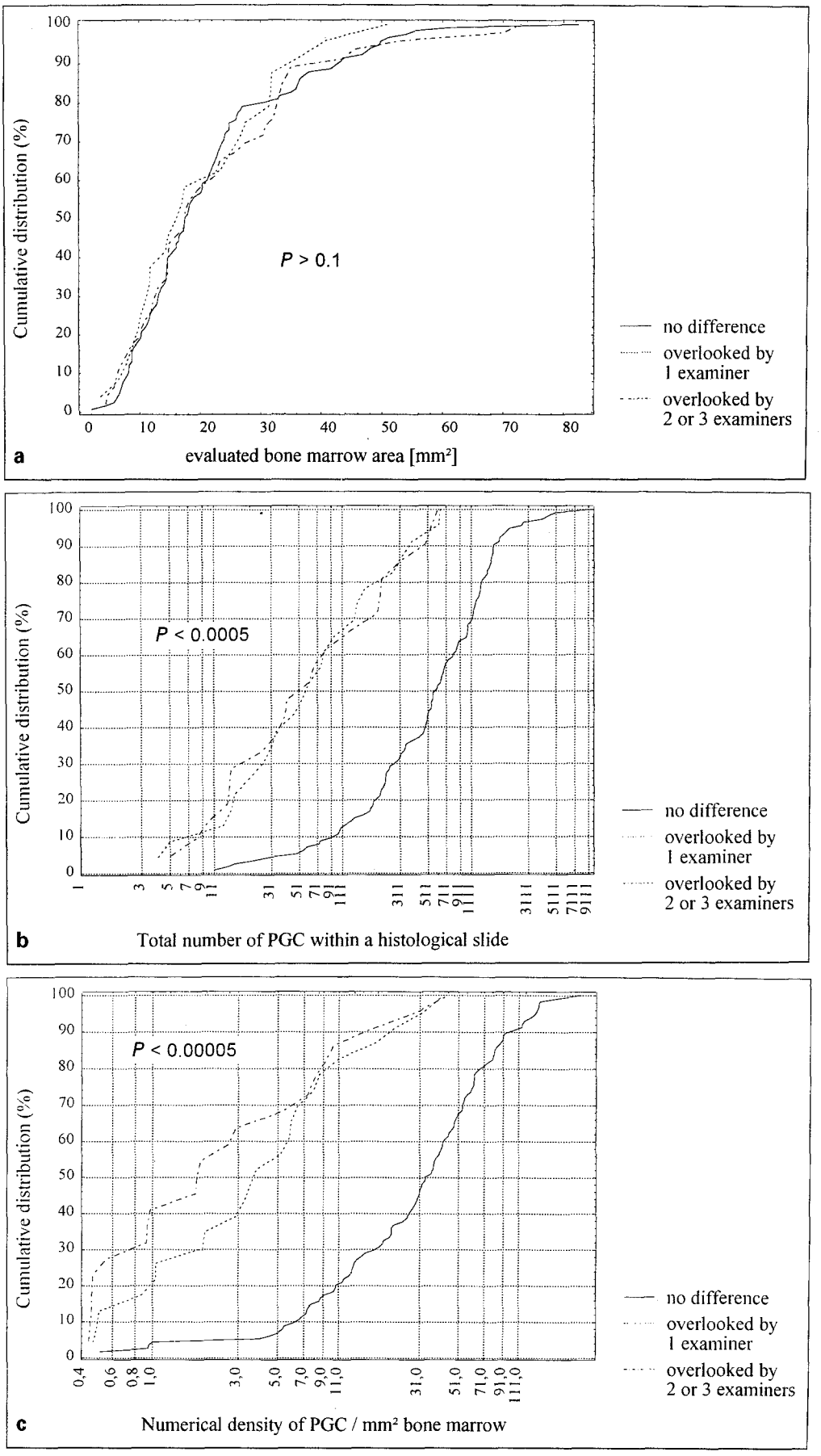

Fig. 2a–c Comparison of missed (n=71) and detected (n=131) PGC-positive cases: The total number and numerical density of PGC were significantly lower in PGC-positive biopsies in which they were overlooked. These differences were highly significant, but it was not important whether a PGC-positive biopsy was overlooked by 1 (n=26), 2 (n=15) or 3 (n=30) examiners ($P > 0.05$). The quality of a biopsy, i.e. the artifact- and bone-free marrow area that could be examined, did not play an important part ($P > 0.1$)

B, C, E and G (Table 3). Another important influence was the time taken for the investigation: observer B overlooked the PGC in 39% (33/85) of PGC-positive BMB when his evaluation took less than 20 min per slide, and in 19% (15/77) when he took more than 20 min per slide ($P<0.01$). The detected frequency of PGC-positive cases was not substantially influenced by the method of embedding (Table 6). However, the time the observers needed for the investigation was about 50% longer when slides from paraffin-embedded BMB were examined.

The birefringence was highly specific for PGC: 172/203 BMB with birefracting histiocytes showed this feature only within the cytoplasm of PGC, and 29 of the remaining 30 sections showed a significant proportion of typical PGC among the birefracting histiocytes, the others being histiocytes with overlapping features between PGC and sea-blue histiocytes or histiocytes with cell debris (Table 2). The birefringence was not detectable in normal histiocytes with or without cell debris, in sea-blue histiocytes or in histiocytes with green crystals within the cytoplasm, which were also detected in a significant proportion of the BMB from the CML patients (Table 2).

Observer disagreement and frequency of PGC-positive cases

The percentages of "positive" findings in the BMB from untreated patients with Ph-positive CML varied around 50% (40–58%; Table 3). Observers D and F used different microscopes. Examiners A, B, C, E and G all used the high-quality microscope II and no differences could be detected between the patients recorded in the Bone Marrow Registry, Hannover (observer G) and the patients recruited into the German CML Study I (observers A, B, C, E; Table 3). The rate of observer disagreement was not substantially influenced by the method of embedding, because no significant differences were found between plastic and wax preparations (Table 6).

However, a systematic comparison of the individual results (observers A, B, and C) uncovered a high percentage of dissent cases (Tables 4, 5), even when the same microscope had been used. In the critical discussion at the microscope, 41/53 dissent cases were shown to be missed PGC-positive cases, the majority of them showing more than 60 PGC within the slide (Fig. 2 b). A critical revision by two other haematopathologists of such "positive" cases that had been overlooked by at least two examiners confirmed the PGC-positive diagnosis in each case (Table 5). Thus, BMB that were judged as positive but could not be confirmed as such by another examiner accounted for about 1% of cases (Table 4).

In contrast, a systematic review by three examiners of the 117 BMB that were considered "negative" by each observer (A, B and C) revealed an additional high percentage (22%) of clearly positive cases that had been overlooked. Thus, the percentage of PGC-positive cases increased to 69% (Table 5).

Table 7 Comparison of cytological and histological examination: sufficient bone marrow smears were additionally available in 21 cases, and they were examined for PGC with no awareness of the results found in the BMB that had been taken simultaneously. The detected frequency and the total count of PGC found by cytological examination were significantly lower

Technique	Frequency of PGC		Mean PGC count per slide	Significance
	Ratio	%		
Histology (biopsies)	15/21	71.4	588 ± 884	$P<0.04$
Cytology (smears)	8/21	38.1	59 ± 187	

At least 4 typical PGC showing a fibrillar birefringence of their cytoplasm (see Fig. 1) were detected in all PGC-positive BMB that were confirmed in consensus.

Risk of overlooking PGC

The total number of PGC/slide and the frequency of positive cases were significantly lower in bone marrow aspirates than in the BMB that had been taken simultaneously (Table 7). Among the PGC-positive aspirates, the majority of the PGC were usually located within the marrow crumbs.

PGC-positive BMB that were overlooked by at least one examiner also showed a significantly lower total count or numerical density of PGC (Fig. 2). The differences between overlooked and detected PGC-positive BMB were highly significant ($P<0.0005$), but it was not important whether a PGC-positive biopsy was overlooked by one ($n=26$), two ($n=15$) or three ($n=30$) examiners ($P > 0.05$). The cumulative distributions of (a) the evaluable marrow area, (b) the total count of PGC within a slide, and (c) the numerical density of PGC/mm^2 are illustrated with respect to overlooked and detected PGC-positive cases in Fig. 2.

In a few cases of bilateral trephine biopsies, the PGC could be detected in only one of the BMB. However, no influence of the evaluable (artifact- and bone-free) marrow area was detected: large biopsies with a high total count of PGC but a low numerical density were misinterpreted as negative as often as small biopsies with a high numerical density but a low total count of PGC (Fig. 2). Small BMB with less than 10 PGC were rare (10/301 BMB); however, all of them had been overlooked by at least one observer (Fig. 2).

The lower the total number or the numerical density of PGC ($n=202$) and the more pronounced the inhomogeneity of PGC distribution within a slide ($n=72$), the higher the risk of overlooking the PGC. The estimated risks of overlooking are illustrated in Fig. 3. Based on the results from examiners A, B and C, the risk of overlooking was about 80% if the total PGC count was 70 or less, the numerical density was 5 PGC/mm^2 or less, or the coefficient of variation of the PGC distribution exceeded 1.2 (Fig. 3). The risk of overlooking was about

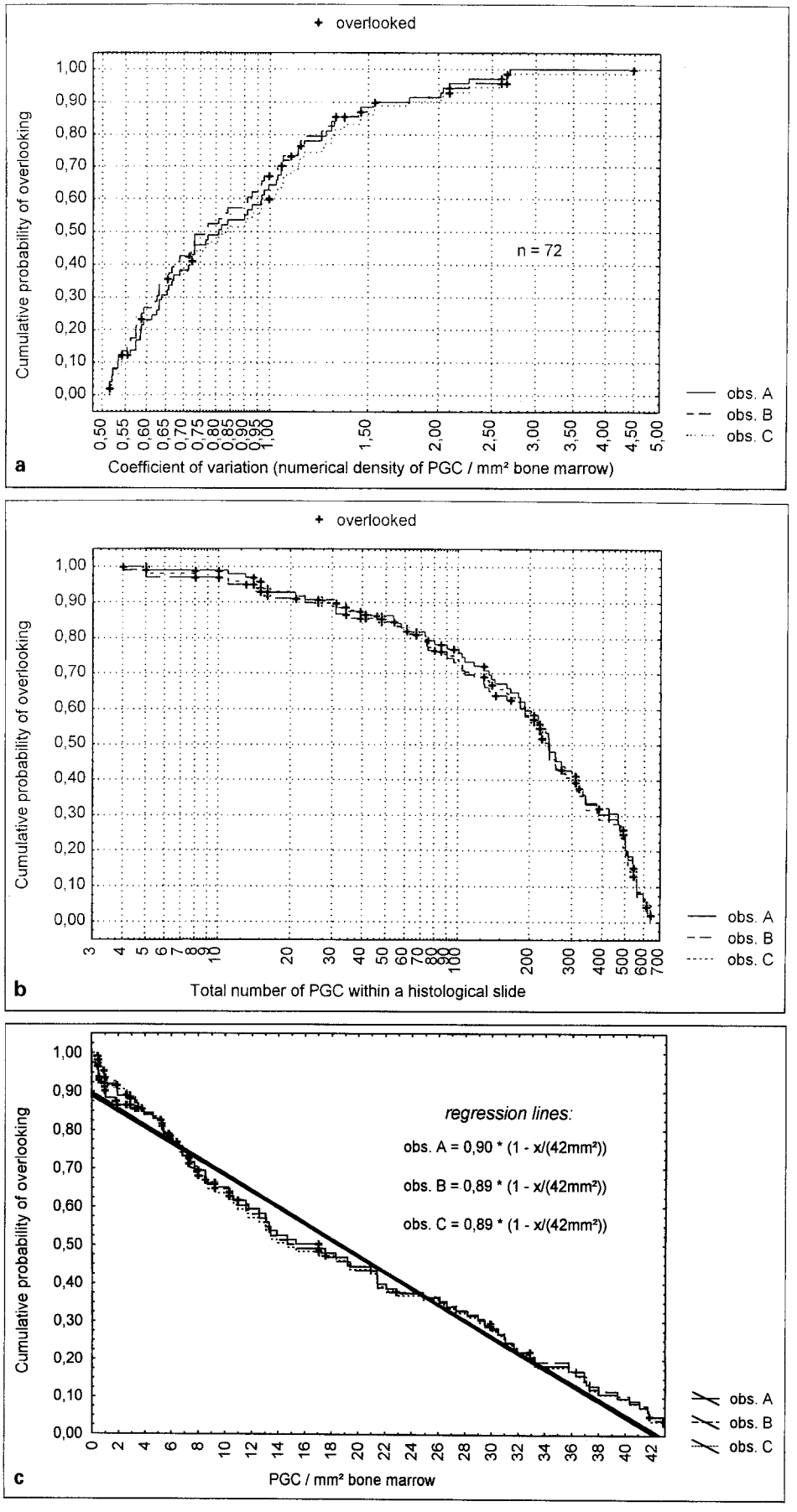

Fig. 3a–c Risk of overlooking PGC: the lower the total number (**b**) or the numerical density of PGC (**c**, n=202) and the higher the inhomogeneity of PGC distribution within a slide (**a**, n=72), the higher the risk of overlooking the PGC. Examiners A, B and C showed almost identical risk curves, although (1) they had examined independently and (2) the majority of overlooked cases were not identical

50% if the total PGC count was 250 or less, the numerical density was 17 PGC/mm^2 or less, or the coefficient of variation of the PGC distribution within a slide exceeded 0.75 (Fig. 3).

No significant differences were detected betweeen the individual risk curves of examiners A, B and C (Fig. 3).

Discussion

PGC are a highly characteristic finding in bone marrow from patients with a Ph-positive CML (Table 1). Since their first description in three decades ago, the occurrence of these histiocytes has been confirmed repeatedly by other laboratory groups, and a prolongation of survival was observed when Gaucher-like histiocytes were detected in the bone marrow [15, 18, 20–22]. However, an enormous variation in the reported frequencies among the authors, ranging from 5% to more than 50% (Table 1), indicates the necessity for a critical analysis of their natural frequency in untreated CML, especially as their occurrence has been introduced into a risk score [22].

Our results, based upon the evaluation of the diagnostic BMB from more than 800 patients with a CML, can be summarized in two statements: The natural frequency of PGC in CML is much higher than assumed to date; and the differences between observers are explained by the method of examination.

One important influence was detected: the count of PGC in bone marrow aspirates was only about one tenth of that in histological sections, which might explain the very low frequencies reported by some authors whose investigations were performed on bone marrow aspirates (Table 1). Obviously, the PGC were retained within the reticulin meshwork. This explanation is supported by the finding that in aspirates the PGC are usually located within the marrow crumbs.

The method used, with light microscopic demonstration of PGC within histological sections by their typical cytomorphology and birefringence of the cytoplasm, turned out to be very sensitive and highly specific. These findings confirm earlier observations from Hayhoe et al. [11] and Takashaki et al. [17], who described the typical birefringence of the cytoplasm in these cells.

Histiocytes with overlapping features between PGC and other storing histiocytes were not a real problem, because almost all of these cases also showed a significant count of typical PGC, and histiocytes with overlapping features between PGC and others might be early stages of PGC. Furthermore, when a high-quality microscope is used, the exclusion of histiocytes with a Gaucher-like but not birefringent cytoplasm does not result in a significant loss, but the rate of observer agreement can be improved.

From these results it is clear that other methods of light microscopic marking of the PGC, such as Sudan staining or marking with GSA-I lectin [2, 11, 17], seem to be indicated only if a polarization microscope of high quality is not available. The notable disadvantage of these alternative methods is the impossibility of distinguishing between PGC and the other types of storing histiocytes in CML, especially sea-blue histiocytes. These storing cells can also be detected in the bone marrow from patients with CML, but they are not as specific as the PGC [7] because they are also usually found in Ph-negative leukaemic and preleukaemic disorders, especially in myelodysplastic syndromes [14].

In spite of the good agreement with respect to the frequency of PGC-positive cases among the seven observers, who had worked independently, a systematic analysis of the results revealed a high rate of missed PGC-positive cases. The risk of overlooking PGC was significantly influenced by the total count and the numerical density of PGC as well as the inhomogeneity of PGC distribution within the slide. The overlooked positive cases could not be ignored, because overlooked cases with few PGC (fewer than 10) were rare: the risk of missing the cells went down to 5% only when the total count of PGC within a slide exceeded 600. Overlooking is a general problem: the three examiners, A, B and C, whose results were tested systematically showed almost identical risk curves although they had worked independently.

Thus, the natural frequency of the occurrence of PGC in Ph-positive CML is much higher than assumed hitherto, amounting to about 70%. The rather low percentages reported in the literature may be explained by the method of examination (aspirate versus biopsy) and by the high risk of individuals' overlooking these histiocytes. The surprisingly high natural frequency might be due to several factors: the evaluation of biopsies, the careful and systematic examination at high magnification, application of the very sensitive polarization using a high-quality polarization microscope and, finally, an effective control of the individual results by a panel of several examiners, with critical discussion of dissent results and re-evaluation of positive and negative cases.

The question arises of how reliable data on the natural distribution of these cells in the bone marrow of untreated patients can be obtained. (1) A clear definition of positivity must be given. In our own experience, 4 unequivocal, characteristic PGC must be shown, all of which reveal fibrillar birefringence of their cytoplasm. (2) A polarization microscope of high quality should be used, and (3) a single examination performed by one observer is insufficient even when the examination time extends to 20 min per slide: scientific examinations should be performed by a panel of several examiners with discussion of dissent results and additional re-evaluation of cases initially judged as negative. As estimated by a statistical approximation based on the observed risk of overlooking PGC (see Appendix), a deviation from the real rate of PGC-positive cases in Ph-positive CML lower than 5% can be expected only with such a labour- and therefore cost-intensive evaluation

Four questions arise from this surprisingly high occurrence of PGC-positive bone marrow samples from untreated CML patients. These are: 1. Are PGC really important in the course of CML? 2. Does the influence depend on the quantity (the numerical density) of PGC? 3.

Table 8 Frequency of PGC among untreated CML patients estimated by analysis of the risk of overlooking (*n* number of examinations; R_n estimated risk of overlooking)

Statistical approach		Frequency of PGC	
n	1-R_n	Estimated	Observed
1[a]	0.55	48.6%	49.8–52.2%
2[b]	0.73	58.2%	57.0–58.0%
3[c]	0.82	62.8%	60.0%
6[d]	0.92	68.9%	68.9%
12	0.98	71.3%	
∞	1.00	72.4%	

[a] BMB misinterpreted as positive were considered negative
[b] Dissent cases were excluded
[c] Panel diagnosis
[d] Revision of panel results

Does the numerical density of PGC change during the course of disease and does any change depend on the therapy applied? 4. What is the importance of PGC for the differential diagnosis?

These questions will soon be answered by evaluating patients enrolled in the German CML Study.

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Appendix *A*: Statistical estimate of the "real" frequency of PGC in the bone marrow from untreated CML patients by analysis of the risk of overlooking

If the individual risk curve of overlooking PCG in biopsies with a numerical density x ≤ 42 PGC / mm² is known, then the rate of positive findings among *n* independent examinations is binomial distributed with $B(n;1-r_x;k)=\binom{n}{k}(1-r_x)^k r_x^{(n-k)}$, $0 \le k \le n$ and r_x=the probability of overlooking the PGC within a slide with x PGC / mm² and x ≤ 42 PGC / mm². The probability of only negative findings, i.e. k=0, results in r_x^n.

If each value $0 < x \le 42$ / mm² is possible without significant differences between the probabilities of lower and higher values, then the mean risk of overlooking the PGC in all of n examinations is $R_n = \int_0^{42/\mathrm{mm}^2} r_x^n dx$ with $0<x\le 42$ / mm².

In the present example, the risk curve r_x was estimated by the regression line from the observed risk of overlooking (see Fig. 3c) with $r_x = 0.9\left(1-\frac{x}{42/\mathrm{mm}^2}\right)$. The mean risk of overlooking PGC was calculated as follows:

$$R_n = \int_0^{42/\mathrm{mm}^2} r_x^n dx = \int_0^{42/\mathrm{mm}^2} \left(0.9\left(1-\frac{x}{42/\mathrm{mm}^2}\right)\right)^n dx$$
$$= 0.9^n \int_0^1 (1-y)^n dy = \frac{0.9^n}{n+1}.$$

The final evaluation, the revision of the panel diagnosis, was based upon a total of six examinations (three primary and three re-examinations). According to the statistical model presented, the remaining risk of overlooking was estimated as $R_6 = \int_0^{42/\mathrm{mm}^2} r_x^6 dx = \frac{0.9^6}{7} = 0.076$ (which is an approximation because the revision of the panel diagnosis was not performed independently of the previous examinations).

The frequency of BMB with $x > 42$ /mm² was 19.6% (59/293), and the observed rate of BMB with $0 < x \le 42$ / mm² was 48.8% (143/293). Considering the risk of overlooking, the "real" frequency of BMB with $0 < x \le 42$ / mm² is estimated approximately as $48.8\%\frac{1}{1-R_6} = 52.8\%$, which is 4% higher than the observed rate. Thus, applying this statistical model, the detected frequency of PGC-positive cases can be estimated at 52.8% (1–R_n) + 19.6% depending on the number of examinations (n).

The frequencies estimated by this model agreed well with the frequencies actually observed (Table 8) indicating that the approximation by this model is adequate. Therefore, it can be assumed, that the "real" frequency of PGC in untreated patients with Ph-positive CML is about 70–75%.

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