Smudge cells in peripheral blood smears did not differentiate chronic lymphocytic leukemia from other B-cell chronic lymphoproliperative diseases

Sombras nucleares no esfregaço do sangue periférico não diferenciam a leucemia linfocítica crônica das outras doenças linfoproliferativas B crônicas

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Smudge cells has been classically associated with chronic lymphocytic leukemia (CLL), but they are found in peripheral blood tests for other chronic B-cell lymphoproliferative diseases (CLD). We investigated whether the percentage of smudge cells in peripheral blood smears can be used in the clinical practice to differentiate CLL from other B-cell CLD. The peripheral blood smears of 63 patients with the diagnosis of CLL and 62 with other B-cell CLD were analyzed. Three hundred cells (both lymphoid cells and smudge cells) were counted for each peripheral blood smear. A comparison of the percentage of smudge cells between the two groups was performed and, subsequently, 5 cut-off values were fixed (10%, 20%, 30%, 40% and 50% of smudge cells) with the aim of defining cases as "positive" or "negative" for smudge cells and verifying whether there are any differences between CLL and the other B-cell CLD. The percentage of smudge cells in patients with CLL (median 26%, 4%-86%) was higher than in patients with B-cell CLD (median 14%, 1%-64%). However, none of the cut-off values tested presented suitable values of sensitivity, specificity and positive predictive value to separate the two groups. As it is necessary to have a single cut-off value with high sensitivity, specificity and positive predictive value to infer the diagnosis of CLL in the clinical practice, we concluded that smudge cells are not fitting to differentiate CLL from other B-cell CLD. Rev. Bras. Hematol. Hemoter.

Key words: Chronic lymphocytic leukemia; B-cell lymphoproliferative diseases; smudge cells; flow cytometry.

Introduction

Smudge cells, also known as Gumprecht cells[1] are ragged lymphoid cells found mainly in peripheral blood smears of patients with chronic lymphocytic leukemia (CLL) and other B-cell chronic lymphoproliferative diseases (CLD). Smudge cells are not simply artifacts of slide preparations and there is no correlation between absolute lymphocyte count and the percentage of these cells.[2]

Therefore they may be related to the biological characteristics of leukemic cells, as demonstrated in a recent paper where low vimentin expression could render CLL cells more susceptible to smudging when making peripheral blood smears.[3]

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Although smudge cells can be found in different B-cell CLD and even in reactive lymphocytosis,[4] they have been classically associated with the diagnosis of chronic lymphocytic leukemia. In fact, even in recent publications the smudge cells are considered a typical finding which strongly suggests the diagnosis of chronic lymphocytic leukemia.[5]

In this study, we investigated if the percentage of smudge cells in peripheral blood smears could really be used in the clinical practice of hematology to differentiate CLL from other B-cell CLD.

Design and Method

Patients

We analyzed the peripheral blood smears of 125 patients with the diagnosis of mature B-cell neoplasm: 63 with chronic lymphocytic leukemia and 62 with other B-cell chronic lymphoproliferative disorders (31 with mantle-cell lymphoma, 11 with splenic marginal B-cell lymphoma, 5 with B-cell prolymphocytic leukemia, 5 with follicular lymphoma, 3 with lymhoplasmacytic lymphoma, 1 with hairy cell leukemia and 6 patients with a B-cell lymphoproliferative disorders not otherwise categorized). All cases of CLL had 4 or 5 points in the Matutes scoring system,[6] while all other CLD cases had 0, 1 or 2 points. We excluded all patients with 3 points in the scoring system. The patients' diagnosis was based mainly on morphological and immuno-phenotypical features. All mantle-cell lymphoma cases had evidence of CYCLIN D1 overexpression measured by real-time polymerase chain reaction (data not shown). All peripheral blood slides belong to the "Blood and Bone Marrow Bank" of the Laboratory of Hematology, University of Sao Paulo, which was approved by the Ethics Commitee of University Hospital, School of Medicine of Ribeirao Preto.

Morphological examination and immunophenotyping

The blood smears of all samples were prepared from either EDTA anticoagulated blood or a finger stick, by manual wedge method, in which a drop of blood is pulled by a glass cover. Three-hundred cells, among lymphoid cells and smudge cells, were counted simultaneously by two hematologists on each peripheral blood smear stained with Leishman stain, and 10% of the samples were randomly selected to be blindly reviewed by a third independent investigator. All samples were considered to be consistent with the diagnosis of a mature B-cell neoplasm on morphologic examination.

Mononuclear cells were isolated from peripheral blood samples by Fycoll Hypaque density gradient centrifugation. The following panel of monoclonal antibodies directly conjugated with fluorescein isothiocyanate (FITC) or phycoerythrin (PE) was used: CD2, CD5, CD10, CD19, CD23, CD79b, CD103, FMC7, Kappa/Lambda-immunoglobulin light chain. All monoclonal antibodies were purchased from Becton Dickinson, except FMC7 (Dako, USA). All samples were analyzed with a FACScan (Becton Dickinson, San Jose, CA). The Cell Quest software was used for data acquisition and analysis.

Statistics

The samples did not present a normal distribution based on the Kolmogorov-Smirnov test performed with the SPSS 9.0 software. So, the comparison of the percentage of smudge cells between the two groups (CLL vs. B-cell CLD) was performed by the Mann-Whitney test, two-tailed, using GraphPad 4.0 Prism software. Subsequently, we fixed 5 cut-off values of more than 10%, 20%, 30%, 40% and 50% of smudge cells with the purpose to define a case as "positive for smudge cells" and verify if there were any differences between CLL and the others CLD. We used a Fisher's exact
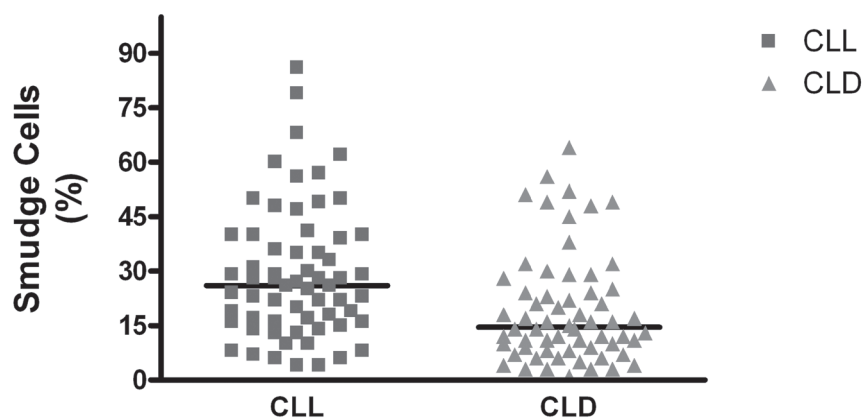

Figure 1. Median (horizontal bars) of percentage of smudge cells between chronic lymphocytic leukemia (CLL) and other B-cell chronic lymphoproliferative diseases (CLD)

Table 1. Frequency of smudge cells in CLL and CLD cases with five different cut-offs

	Percentage of Smudge Cells														
	<50%	≥ 50%		<40%	≥ 40%		<30%	≥ 30%		<20%	≥ 20%		<10%	≥ 10%	
			p* value			p* value			p* value			p* value			p* value
CLL	54/63	9/63	0,2408	47/63	16/63	0,1110	40/63	23/63	0,0459	22/63	41/63	0,0023	7/63	56/63	0,0242
CLD	58/62	4/62		54/62	8/62		50/62	12/62		39/62	23/62		17/62	45/62	

test, and a p value of less than 0.05 was considered of statistical significance.

Results

The percentage of smudge cells in peripheral blood smears of patients with the diagnosis of chronic lymphocytic leukemia [26% (range, 4%-86%)] was higher than in patients with B-cell chronic lymphoproliferative disorders [14% (range, 1%-64%)] (p=0.0005) (Figure 1). We then analyzed the discriminating capacity of the five different cut-offs. Table 1 show that percentages of smudge cells equal or higher than 10%, 20% and 30% were found to generate two subgroups with frequencies of CLL cases statistically different. We determined the variables related to the tests (sensitivity and specificity) and the positives predictives values in order to verify if the separation between the CLL and the others CLD could really be possible on clinical grounds (Table 2).

Table 2. Sensitivity, specificity, positive predictive value and confident intervals of three

	30% cut-off		20% cut-off		10% cut-off	
Sensitivity	36%	25%-49%*	65%	52%-77%*	89%	78%-95%*
Specificity	81%	68%-89%*	62%	50%-75%*	27%	17%-40%*
Positive predictive value	65%	48%-81%*	64%	51%-76%*	55%	45%-65%*

*Confidence Interval 95%.

Discussion

The presence of smudge cells has been classically associated with the diagnosis of chronic lymphocytic leukemia. In this study, we investigated the presence of smudge cells in 125 randomly selected patients with mature B-cell neoplasms, with the purpose to verify if the widespread belief that smudge cells are more frequently associated with the diagnosis of chronic lymphocytic leukemia is a true conception.

Thus, three cut-offs were found to be able, in theory, to separate CLL patients from CLD patients: 10%, 20% and 30%. Since these values have allowed a statistically valid separation between the two groups, we have considered which one of them as a "test" to be used in the differentiation between CLL and CLD. We calculated the sensitivity and the specificity of 10%, 20% and 30% cut-offs, since these pre-test variables are the most important determinants of the utility of any diagnostic test intended to be used in clinical practice. Moreover, we calculated the positive predictive value because this post-test probability is, in fact, the value used by clinicians in their diagnosis decision process (Table 2).

The cut-off of 10% has presented the higher sensitivity (89%), and the cut-off of 30% has presented the higher specificity (81%) for the diagnosis of CLL. However, the cut-off of 10% has showed a low specificity (27%), and the cut-off of 30% has showed a low sensitivity (36%). The cut-off of 20% has presented intermediate values of sensitivity and specificity (65% and 63%, respectively). Thus, for each one of the three "tests", the probability of be positive (percentages of smudge cells higher than the cut-off) provided that the diagnosis is CLL, and, at the same time, the probability of be negative (percentages of smudge cells lower than the cut-off) provided that the diagnosis is not CLL are not sufficient to precisely separate between the CLL and the others CLD. This occurs because there is an overlay of smudge cells' percentages in both CLL and CLD. Besides, the probability that a patient has the diagnosis of CLL provided that the test is positive (positive predictive value) based on that three cut-offs is low and also not sufficient to distinguish the two groups.

Hence, even if the percentage of smudge cells in CLL was higher than in patients with CLD when we compared directly the two groups, it would be necessary to have a single cut-off value with higher sensitivity, specificity and positive predictive value to infer the diagnosis of CLL in clinical practice.

When using a test as a diagnostic tool, the validity of the test, measures by the rates of sensitivity and specificity, is what gives the information about the value of the test for its clinical application. Smudge cells on peripheral blood smears are, at most, "statistically significant", but, essentially, they are not "clinically significant" to discriminate CLL from other CLD.

In clinical medicine, it is very common that a reasonable and general assumption among physicians become

considered a really proved medical information. These "widespread beliefs" occur mainly because they make some pathophysiologic sense, but they are infrequently submitted to clinical studies.[7] The results of this report show clearly that, on clinical grounds, the percentage of smudge cells in peripheral blood smears have no value as a morphologic finding for the differentiation between CLL and others B-cell CLD.

Resumo

As sombras nucleares têm sido classicamente associadas à leucemia linfocítica crônica (LLC), embora possam ser encontradas nos esfregaços do sangue periférico de outras doenças linfoproliferativas B crônicas (DLBC). Nesse estudo, nós investigamos se a porcentagem de sombras nucleares nos esfregaços do sangue periférico pode ser utilizada na prática clínica da hematologia para diferenciar a LLC das outras DLBC. Foram analisados os esfregaços do sangue periférico de 63 pacientes com o diagnóstico de LLC e 62 com outras DLPC. Trezentas células, entre células linfoides e sombras nucleares, foram contadas em cada esfregaço. A comparação da porcentagem de sombras nucleares entre os dois grupos foi realizada e, subsequentemente, foram fixados 5 cut-offs de mais de 10%, 20%, 30%, 40% e 50% de sombras nucleares com o propósito de definir um caso como "positivo para sombras nucleares" e verificar se havia diferenças entre a LLC e as outras DLBC. A porcentagem das sombras nucleares em pacientes com LLC (mediana 26%, 4%-86%) foi maior do que em pacientes com DLBC (mediana 14%, 1%-64%). Entretanto, nenhum dos cut-offs testados apresentou valores apropriados de sensibilidade, especificidade e valor preditivo positivo para distinguir os dois grupos. Desde que é necessário se dispor de um único valor de cut-off com alta sensibilidade, especificidade e valor preditivo positivo para inferir o diagnóstico de CLL na prática clínica, conclui-se que as sombras nucleares não são úteis para diferenciar a LLC das outras DLBC. Rev. Bras. Hematol. Hemoter.

Palavras-chave: *Leucemia linfocítica crônica; doenças linfoproliferativas B; sombras nucleares; citometria de fluxo.*

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