Smudge Cells: What Technologists Need To Know

Summary: Although smudge cells have been identified in patients with chronic lymphocytic leukemia since 1896, the mechanism of their formation is still not known with certainty. Smudge cells are common artefacts, and may be formed from any white cell in the peripheral blood film. Their presence in large numbers suggests an abnormally high mechanical fragility of leukocytes resulting in their traumatic disintegration during blood film preparation. This article describes the mechanism of smudge cell formation in the peripheral blood film, particularly in chronic lymphocytic leukemia. Criteria for reporting smudge cells are also presented.

Introduction

Distinguished by their naked amorphous nuclear chromatin material, smudge cells were initially described by Gumprecht in 1896 as white blood cells with broken-down nuclei (Gumprecht’s nuclear shadows) in patients with chronic lymphocytic leukemia (CLL). Subsequently, these nuclear shadows have been referred to as smudge cells, basket cells, dead cells, degenerated cells, disintegrated cells, damaged cells or crushed white cells. The mechanism by which smudge cells appear in the peripheral blood film (PBF) is not completely understood but has been associated primarily with the traumatic disruption of cells during blood film preparation. In the process, the cell wall ruptures, and when seen under the microscope, what remains looks like a smudge, hence the term “smudge cells”. Smudge cells are thus nuclear remnants of leukocytes (usually lymphocytes) with structureless chromatin material. Because they are intact cells both within the circulation and in the blood sample, they are counted as whole cells in the automated leukocyte count.

Although smudge cells can be observed occasionally in normal PBFs, they can also be seen in variable numbers in abnormal PBFs, e.g., in cases of acute myeloblastic leukemia, chronic myelocytic leukemia, monocytic leukemia, acute lymphoblastic leukemia, and usually in greater numbers in chronic lymphocytic leukemia. Smudge cells being common artefacts, they may result from any white cell in the PBF. In normal PBF, there may be 0 to 1% smudge cells. An early study showed the mean to be 5% (range 0-12%). This value invariably increased to greater than 10% and sometimes greater than 50% or more than 100 smudge cells per 100 leukocytes in CLL.

CLL is characterized by a progressive accumulation of clonal B-lymphocytes. These typically resemble mature small lymphocytes with a conspicuous though narrow rim of cytoplasm. Other CLL lymphocytes are atypical: the cells may have cleaved nuclei, or they may be large with abundant cytoplasm and characteristic features, e.g., in prolymphocytic leukemia, large granular cell leukemia, and T-CLL cells. CLL cells often smudge when blood films are made, probably because they are more fragile than normal cells.

The exact mechanism of smudge cell formation in CLL has not been completely elucidated until now. The process depends on a complex physical interaction between the blood cells and glass slides during blood film processing. It appears that the most common condition associated with large numbers of smudge cells is CLL.

In typical CLL, the nucleus virtually occupies the entire cell leaving only a thin rim of visible cytoplasm. Unlike other white cells, the lymphocytes of CLL are not flattened easily when subjected to external pressure, and are crushed directly during the film preparation forming smudge cells. CLL lymphocytes may show certain basic structural abnormalities at the molecular level. Microtubules appear to be involved in the surface capping phenomenon of immunoglobulin of normal lymphocytes. It is possible than microtubules have been called the skeleton of the cell, an abnormal cytoskeletal structure of CLL lymphocytes may predispose them to smudging.

Furthermore, CLL lymphocyte membranes possess various alterations in the expression of certain antigenic determinants. There is altered expression of A, B, H and HLA antigens. A decrease in galactose, N-acetylgalacosamine, mannos, and sialic acid content, and changes in lipid content are also observed. The changes in membrane glycolipids and glycoproteins, and in phospholipid metabolism seem to be responsible in part for the observed weakness of the lymphocyte membrane. Moreover, impaired pentose phosphate shunt and abnormal glycogen metabolism are also noted, presumably due to the lack of enzymes. If these variations are compared to the changes observed in the red cell enzymes and metabolic processes during aging, they may signify senescence of the cells. In CLL, these marked alterations coupled with abnormal cytoskeletal structure may translate into structural fragility – the so-called smudge-cell phenomenon.

Recently, the increased interest in apoptosis (regulated process of programmed cell death) has led to investigations into the role of dysregulation (defect in induction) of apop-
tosis as a mechanism for the prolonged cell survival of B-CLL cells in the blood.\textsuperscript{20,21} Apoptosis is an important control mechanism for eliminating surplus or unneeded cells without inducing an immune or inflammatory response. An organism requires that some of its cells die on schedule so that others may live in more healthy homeostatic conditions. This process of cell death must be coordinated and regulated so as to protect the overall well-being of the organism.\textsuperscript{5,21,22}

The definitive cause of the dysregulation of apoptosis in CLL remains unclear.\textsuperscript{20,23} What is clear, however, is that this defective apoptosis plays a pivotal role in the relentless accumulation of malignant cells that fail to die; hence, the increased numbers of lymphocytes in circulation as opposed to increased cell proliferation.\textsuperscript{24} Moreover, it is interesting to note that these malignant cells, which are in an advanced stage of maturation, have the propensity to undergo apoptosis “cytotoxic” in vitro.\textsuperscript{21,23} As a result, these peculiar characteristics of CLL lymphocytes (small rim of cytoplasm, abnormal cytoskeletal structure and cell membrane, and increased accumulation of older cells in the blood) predispose them to smudging during film processing. This may explain why there is an increased number of smudge cells observed in CLL.

Factors That May Influence Smudge Cell Formation in Blood Films

The factors that influence the formation of smudge cells in the blood films are complex but may be classified as shown in Table 1. Three major factors have been defined, based mainly on the in vitro observations of cell disintegration during blood film preparation. Although it is useful to attribute the condition to one process, it is important to understand that multiple interrelated factors contribute to the proportional increased or decreased numbers of smudge cells in the PBFs.

1. Preparation of blood films. The proportion of smudge cells seen on the blood films can vary widely, due in part to the expertise with which films are prepared. Preparing adequate blood films exposes the cells to mechanical trauma during the spreading process.\textsuperscript{6} This spreading process influences the quality of the blood film and the numbers of smudge cells formed. If the angle of the slide spreader is high (between 40 and 60 degrees) and the speed of spreading the blood is fast, a thick film may be formed with a more uniformly dispersed cell population.\textsuperscript{2,25} The opposite happens if the specimen is spread slowly, with more pressure and at a low angle (between 20 and 30 degrees). This, along with a sharp-edge slide spreader, will result in a thin well-spread blood film but with relatively more smudge cells than in the thick blood film. A properly made and stained wedge-type blood film should be used for the differential cell count. A smooth clean film. A properly made and stained wedge-type blood film

A. Angle (degrees) of push-slide spreading
B. Pressure and speed of push-slide spreading
C. Sharpness (sharp vs smooth edge) of push-slide spreader

2. Morphology of CLL Lymphocytes. Based on the proportion of atypical lymphocytes (prolymphocytes) in the blood, the French-American-British classification system proposed three morphologic CLL types.\textsuperscript{8} These types include:

1) Typical CLL, in which more than 90% of the lymphocytes are small mature-appearing cells with narrow rim of cytoplasm. The nuclear chromatin is dense, and nucleoli are not visible.

2) Mixed cell types, CLL-prolymphocytic leukemia (CLL/PL), in which the percentage of prolymphocytes is greater than 11% but less than 55%. The small lymphocytes of CLL/PL tend to have larger volume than those of typical CLL, and the prolymphocytes are more pleomorphic than those of the prolymphocytic leukemia.

3) Atypical CLL, in which there is a spectrum of small to large lymphocytes but the percentage of prolymphocytes is less than 10%. The lymphocytes are larger than those seen in typical CLL and have increased cytoplasmic basophilia and lower nuclear:cytoplasmic ratio.

These criteria are arbitrary because there are other atypical cases of CLL and lymphoproliferative disorders that should be carefully considered before atypical CLL is diagnosed.\textsuperscript{29} Immunophenotyping of cells and cytogenetic analysis are becoming increasingly helpful in the investigation of lymphoid malignancy and characterizing the nature of the malignant cells. These topics are beyond the scope of this article.

While some authors classify CLL on the basis of cytology and membrane phenotype,\textsuperscript{8} CLL lymphocytes in this discussion are classified according to the size of their cytoplasm as either small or large lymphocytes. This is an arbitrary classification since lymphocytes of intermediate sizes are always present as well. The distinction between small lymphocytes suspended in anticoagulated blood are spherical in shape. They transform into flat cells when spread on the slide due to the surface tension effect of the glass and the evaporation of plasma following film preparation.\textsuperscript{7} Cells in the thin areas where the plasma evaporates quickly lose plasma protein protection\textsuperscript{27,28} and are flattened more than the cells in thick areas where there is little space to spread. Because the cells in the thick areas are bathed in greater amounts of plasma, the cells shrink due to loss of water in the hypertonic plasma. The cells’ diameter may reduce to half that of similar cells in thin areas. Consequently, the cells remain relatively intact and fewer or no smudge cells are observed in this area.

2. Preparation of blood films
A. Angle (degrees) of push-slide spreading
B. Pressure and speed of push-slide spreading
C. Sharpness (sharp vs smooth edge) of push-slide spreader

2. Morphology of CLL lymphocytes
I. CLL lymphocytes with scanty cytoplasm - preponderance of small-sized lymphocytes (<2 red blood cells size)
A. Reactive or viral-like lymphocytes
B. Prolymphocytic leukemia
C. CLL, mixed cell types (a mixture of I and II)
D. T cell CLL/large granulated lymphocyte with abundant cytoplasm containing conspicuous granules

3. Chemistry of plasma proteins
When to Report Smudge Cells

Smudge cells may appear in variable numbers in normal blood films. However, they should be reported only in probable CLL patients, where their increased numbers are noteworthy. The minimal number of smudge cells appropriate to report has not been clearly defined. Some authors/laboratories report smudge cells if there are more than 5 per 100 leukocytes while others report smudge cells when they observe more than 10 to 20 per 100 leukocytes. Early studies show an average high normal range of 10 per 100 leukocytes in probable CLL patients. This is particularly so if the method of reporting results is by number per 100 leukocytes. This results in a significant decrease in absolute lymphocytes (remember lymphocytes are transformed into smudges) and a corresponding relative increase in neutrophils in the differential. Consequently, there would be a significant difference in the automated versus manual differential. In this case, differentials should be verified in the thick areas of the film where 100% of the red cells are overlapping. An alternative method is to include the number of smudge cells in the differential as per cent or absolute count, as suggested by QMP-LS. Otherwise, the leukocyte differential should be performed on a film prepared in the presence of albumin (a 1:5 dilution of 22% albumin and whole blood).

Before reporting smudge cells, a technologist should initially consider the absolute lymphocyte counts of the patient. While hypogammaglobulinemia has been reported in about 8% of cases at the time of initial CLL diagnosis, it may increase up to 65% with disease progression. The significance and influence of this condition on smudge cell formation remains undetermined.

Discussion

To ensure the reliability of results, it is important to understand the effects of variables associated with smudge cell formation, particularly the blood film preparation. This is because their formation is primarily associated with this process. Thus, the angle and the degree of incline of the slide spreader, the type of slide spreader (sharp or smooth), the cleanliness of the slides, and the overall quality of the blood films cannot be over-emphasized. In addition, for minimal morphologic alterations, blood films should be made within three hours and not more than twelve hours after collection.

The suggested criteria for reporting smudge cells (Table 2) are based on many studies and recommendations of different authorities in hematology. This author suggests reporting smudge cells if there are more than 10 per 100 leukocytes in probable CLL patients. This is because smudge cells are characteristically suggestive of this disease.

The grading of smudge cell numbers by the slide-based method is subject to potential variability due to the differences in expertise of making blood films and the areas where differentials are performed on the PBF. For this purpose, and regardless of the methods of blood film preparation (automated vs. manual), differential counts should be performed in areas of the film where there is approximately a 10 to 50% red cells overlap. Performing differentials in the thinner areas near the featheredge of the film (between 0 and 10% red cells overlapping), may produce a relatively higher smudge cell count than in the thicker areas of the film (between 50 and 100% red cells overlapping).

It is important to note that inaccurate differential counts may occur if they are based only on the well-preserved cells. This is particularly so if the method of reporting results is by number per 100 leukocytes. This results in a significant decrease in absolute lymphocytes (remember lymphocytes are transformed into smudges) and a corresponding relative increase in neutrophils in the differential. Consequently, there would be a significant difference in the automated versus manual differential. In this case, differentials should be verified in the thick areas of the film where 100% of the red cells are overlapping. An alternative method is to include the number of smudge cells in the differential as per cent or absolute count, as suggested by QMP-LS. Otherwise, the leukocyte differential should be performed on a film prepared in the presence of albumin (a 1:5 dilution of 22% albumin and whole blood).

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### Table 2. Suggested Criteria for Reporting Smudge Cells

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<td><strong>Absolute lymphocyte count should be greater than 5.0 x 10⁹/L.</strong></td>
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<tr>
<td><strong>Patient age should be more than 30 years.</strong></td>
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<tr>
<td><strong>Smudge cells should be more than 10 per 100 leukocytes.</strong></td>
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*Although CLL is not often diagnosed in patients under the age of 40, patients over 30 years of age should be considered potentially at risk. CLL is virtually unheard of in patients under 30 years of age. In older people, its prevalence rises steadily.*
Although CLL lymphocytes may appear to be normal mature cells, they are actually post-mature, both functionally and developmentally. Typically, CLL is characterized by a very low mitotic rate and the leukemic cells show the morphological and phenotypic features of an advanced stage of maturation. In short, CLL cells are aged cells. Although they may appear normal (strong), their cytoskeletal structure and cell membrane are abnormal (weak) making them vulnerable to smudging when subjected to push-slide blood film processing.

Recently, QMP-LS recommended including smudge cell counts in the differential as a per cent of smudge cells rather than as the number per 100 leukocytes. Although this recommendation is practical and cost effective, most of the laboratories in Ontario report smudge cells as numbers per 100 leukocytes. Including smudge cells in the differential as per cent or absolute count is more appropriate because smudge cells are actually lymphocyte artefacts. This avoids the need for repeating or verifying abnormal counts by the time-consuming albumin-treated method. The main concern about adopting this method is ensuring the understanding and acceptance of our customers – the practising physicians. There is the risk of misinterpreting this smudge cell count as a new cell type. The need for dialogue and education is obvious. Nonetheless, this method has met with general acceptance by most laboratories in Ontario, Canada. Moreover, regardless of method and the number of smudges present, the reporting of smudge cells should include a qualitative notation of their presence.

Many researchers have attributed the formation of smudge cells to the mechanical fragility of the lymphocytes. Some have thought, however, that they represent an early senescent change in the protein structure of the cells. Others believe that the increased numbers of these cell forms result from disease, chemotherapy, sepsis, or necrotic cell lysis, or that in general they tend to be more conspicuous with a high white cell count. Although high white cell counts may influence smudge cell formation, our findings show that smudge cells are more conspicuous when CLL lymphocytes with a narrow rim of cytoplasm are present (unpublished observation).

Despite publications refuting the value of smudge cell counts as a diagnostic indicator of CLL, this author believes that the presence of these cells in large numbers in probable CLL is characteristically suggestive of the disease and therefore may be of diagnostic value.

In conclusion, the cause of smudge cell formation is due primarily to mechanical trauma during blood film preparation secondary to the structural fragility of the CLL cell membrane. Observing procedural precautions in the preparation of blood films and following appropriate guidelines for reporting smudge cells can enhance the diagnostic value of this hematologic finding.

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Benie T. Constantino

ART, MLT, SH, I(ASCP)

Canadian Medical Laboratories, Mississauga, ON

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