Synovial fluid cells analysis: clinical usefulness and analytical issues

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Abstract

An impaired function of synovial fluid (SF) plays an important role in the development of degenerative joint diseases, due to the decrease of the lubricating property of the fluid. After the anamnestic and physical examination, laboratory analysis of SF is the most important test for the evaluation of articular diseases. SF analysis, particularly white blood cell (WBC) count, is useful for differential diagnosis, follow-up and therapy monitoring of arthropaty and polyarthritis. Various pre-analytical and analytical factors (comorbidities, storage, tube type, sample pre-treatment, manual or automatic method) could affect WBC count accuracy, but there is still a lack of standardization of analysis protocols. At the same time, universally accepted clinical cut-off are not available yet. In this article, we described our experience, proposing a viable analysis protocol for SF-WBC count. Considering the importance of this test for a proper diagnosis, the use of standardized procedures will be essential in the future for the improvement of its clinical usefulness.

Keywords: synovial fluid, joint diseases, white blood cell count, N-acetylcysteine, differential diagnosis.

Riassunto

Un'alterata funzione del liquido sinoviale (LS) può determinare lo sviluppo di malattie degenerative delle articolazioni dovute al ridotto potere lubrificante del liquido stesso. Dopo la valutazione clinico-anamnestica del paziente, l'analisi di laboratorio del LS rappresenta il principale esame per indagare le malattie articolari. In particolare, la conta dei globuli bianchi è utile per la diagnosi differenziale, il monitoraggio e la valutazione dell'efficacia terapeutica nelle artropatie e poliartriti. Tale conta può risentire dell'effetto di alcuni fattori pre-analitici e analitici tra cui la conservazione del campione, il tipo di provetta utilizzata per la raccolta, il pre-trattamento del campione e il tipo di metodo utilizzato per la conta (manuale o automatizzato). Ad oggi, manca un protocollo standardizzato per l'analisi del LS e un valore soglia di significatività. In questo articolo descriviamo la nostra esperienza riportando il protocollo analitico che adottiamo nel nostro laboratorio.

Parole-chiave: liquido sinoviale, malattie articolari, conta leucocitaria, N-acetilcisteina, diagnosi differenziale.

Introduction

The synovial fluid is produced by plasma ultrafiltration from fenestrated subsynovial capillary endothelium into the synovial cavity, where it is enriched with hyaluronic acid and glycosaminoglican secreted by the synovial lining cells. Synovial cells are arranged in a 1-3 cell layer, embedded in a matrix without a basement membrane. In order to synthesize proteins, synovial cells phagocytise debris presented at the fluid-cell interface. The functions of the synovial fluid are to lubricate the joint space and to transport nutrients to the articular cartilage[1]. Protein and immunoglobulin content of synovial fluid is about ¼ of plasma, while electrolytes, glucose and uric acid concentrations are similar to blood. Immunological, mechanical, chemical or infectious damage may alter the permeability of endothelium, leading to inflammatory response[2]. An impaired function of synovial fluid with age or disease may play a role in the development of degenerative joint diseases such as osteoarthritis. Inflammatory joint fluids contain various lytic enzymes that cause depolymerization of hyaluronic acid, which greatly impairs the lubricating ability of the fluid.

Various disorders produce changes in the chemical constituents of the joint fluid and the type of cell population present. Through clinical and laboratory examination of the synovial fluid, joint disorders can be divided into 5 categories: 1) non inflammatory, 2) inflammatory, 3) infectious 4) crystal-associated, 5) hemorrhagic.

After the anamnestic and physical examination, laboratory analysis of synovial fluid is considered the most important test for the evaluation of articular diseases.

Indeed, synovial fluid analysis can also support the differential diagnosis of polyarthritis: white blood cell (WBC) count allows to distinguish rheumatoid arthritis from non-inflammatory diseases such as arthrosis[3]. For this purpose, some cut-offs have been defined: a normal synovial fluid has a WBC count < 200/µL with less than 10% of polymorphonuclear leukocytes (PMN), while a result > 2000/µL with > 75% PMN is suggestive of an inflammatory disease, such as crystalloid synovitis (gout, pseudogout) and autoimmune arthritis (rheumatoid arthritis, psoriatic arthritis)[4]. Moreover, this test can also help clinicians to recognize the coexistence of several types of arthropaty in the same subject. For example, patients with rheumatoid arthritis, sickle cell anemia, erythematosus systemic lupus, crystal-associated arthropaty and neurogenic arthropaty may also develop overlapping septic acute synovitis.

Synovial fluid WBC count is obviously of primary relevance for diagnosis of joint infections, such as septic arthritis and periprosthetic joint infection.

Septic arthritis (SA) is an infection in a joint due to a bacterial, mycobacterial or fungal cause. It is a rare event in the general population (2-6 case per 100.000 people per year), but its prevalence grows up to 70 per 100.000 people per year in patients with rheumatoid arthritis. It constitutes a serious cause of morbidity and mortality and an early diagnosis and a timely treatment are needed for a positive outcome. The most common sign of SA is a swollen, warm and painful single joint with reduced motion, while fever is usually low-grade or absent, especially among elderly, and only 40% of patients present with high-grade fever[5]. Periprosthetic joint infections (PJI) are the most common cause of knee arthroplasty failure and the third most common cause of failure in hip arthroplasty, causing prolonged hospitalization and higher morbidity and mortality[6].

Diagnosis of SA or PJI is often difficult, due to the lack of specific diagnostic tests and heterogeneous presentation in patients. Indeed, it relies on a combination of clinical findings, microbiological culture and laboratory results. Synovial WBC count plays a key role, as it has demonstrated good sensitivity and specificity in the diagnosis of SA and PJI. Although no definitive cut-off values exist, 77% of patients with SA has a synovial WBC more than 100000/µL, while only 5% has less than 50000/µL: so a WBC count of 50000/ µL is suggested as a cut-off for diagnosis of septic arthritis.[4,5] Diagnostic thresholds for PJI are still controversial: Musculoskeletal Infection Society (MSIS) criteria for diagnosis of PJI include synovial WBC > 3000/µL, with a proportion of polymorphonuclear cells (PMN) > 70%, but different cut-offs have been proposed for different puncture sites (e.g. knee and hip) and there is no general consensus.

Specimen collection requirements and stability

The collection of joint fluid is made mostly through needle aspiration (arthrocentesis) and requires a good expertise and an extreme care of operators. So, arthrocentesis should be limited to patients with an undiagnosed effusion or a significant clinical change with prior effusion. Operatively, collection of synovial fluid

must be performed with a sterile disposable needle in order to avoid contamination of the joint as well as contamination of collection with particulates and resident skin flora (including coagulase negative *Sthaphilococcus* species and *Propionibacterium acnes*). Lithium heparin, EDTA and oxalate should be avoided in the collection syringe as anticoagulant, because they could lead to the creation of crystalline artifacts. Furthermore, both heparin and EDTA have antimicrobial properties and should not be used to collect specimens intended for microbiologic cultures.

The total volume of collected fluid depends on the joint involved and the nature of the effusion. A proper volume of sample should be divided in different tubes: 5 mL in a plain tube for chemical analysis, 5 mL in EDTA or heparin tube for microscopic analysis, and > 5 mL in a clean sterile tube for additional microbiologic analysis such as the fungal or acid-fast bacterial cultures[7]. For the microbiological specimens all samples should be maintained at room temperature and sent to the laboratory for culture setup within 24 h. Specimens received after 24 h since collection time should be rejected, in order to avoid a false negative culture due to suboptimal specimen handling.

The tube for chemical analysis should be allowed to clot at room temperature and centrifuged as soon as possible: cells may alter chemical composition of synovial fluid (for example, complement levels). The supernatant can be used to measure rheumatoid factor, antinuclear antibodies, complement, or other molecules. For complement assays, the test must be performed within 2-3 hour after the collection to avoid decay due to high temperature. If the sample could not be analyzed immediately, the fluid should be stored at -70 °C until the examination.

Use of local anaesthetics, prior use of antibiotics, clotting, transport of samples, joint site and co-morbid conditions could affect the accuracy of WBC count. The microscopic analysis of the synovial fluid has to be done within an hour after arthrocentesis (optimum < 30 minutes), as storage for 5-6 hours can reduce the visible cells count converting it from ''inflammatory'' to ''non- inflammatory''. The decrease in WBC count over the time is also associated to the decrease of the neutrophils percentage. Fluids that present a large proportion of mononuclear cells, show a reduction in cell count over time[8,9]. Lipid inclusions may develop in synovial fluid specimen incubated for 48 h, causing misleading results.

The effects of specimen storage on crystals can also lead to misleading results: monosodium urate crystals do not decrease significantly over the first 3 days of storage, but they decrease over a period of weeks (due to refrigeration). Calcium pyrophosphate dihydrate crystals instead decrease after 2-3 days in storage and refrigeration do not preserve them, while apatite crystals appear to be resistant to the dissolution over several weeks[8].

If arthrocentesis results in a dry tap, a few drops of fluid still remaining in the needle can be used for the most essential portions of the examination: Gram stain, bacteriological culture and microscopic examination.

Synovial fluid WBC count: our experience

Clear synovial fluid, without protein inclusions must be counted undiluted or diluted with saline solution.

Thick or purulent samples or with protein inclusions could be treated with hyaluronidase or N- acetylcysteine, which reduce disulfide bound present in synovial fluid, making synovial fluid less viscous in order to ease the analytical process. Previous studies demonstrated very good analytic performance after pre-treatment (with hyaluronidase), while results are more variable in non-treated samples, because of high viscosity, which could lead to unreliable results[4].

In our laboratory, synovial fluid WBC count is performed as descrived below:

1. Dilute sample 1:5 with saline solution (NaCl 0.9%).
2. Dilute sample 1:2 with N-acetylcysteine (100 mg/mL).
3. Wait 5 minutes.
4. Count dilute sample in Burker count chamber.

At the same time, automatic cell count is performed using Sysmex XN- 9000 analyzer (Body Fluid mode), which is able to differentiate polymorphonuclear cells and mononuclear cells.

If requested by clinicians, morphological evaluation of synovial fluid is carried out by trained staff in hematology laboratory section.

Some data from our Institution

From January 2018 to November 2019, our laboratory analyzed 83 synovial fluid samples. Of these, 24 samples (28.9%) were sent from Emergency Department, 18 (21.7%) from Pediatric Department, 9 (10.8%) from Haemostasis and Thrombosis Unit and the remaining

32 (38.6%) samples came from various departments of our hospitals. (Figure 1).
Age of patient ranged from 1 to 96 years old, with a median value of 59 years. (24 patients < 18 years old, 27 aged 18-70 years old, 32 > 70 years old).

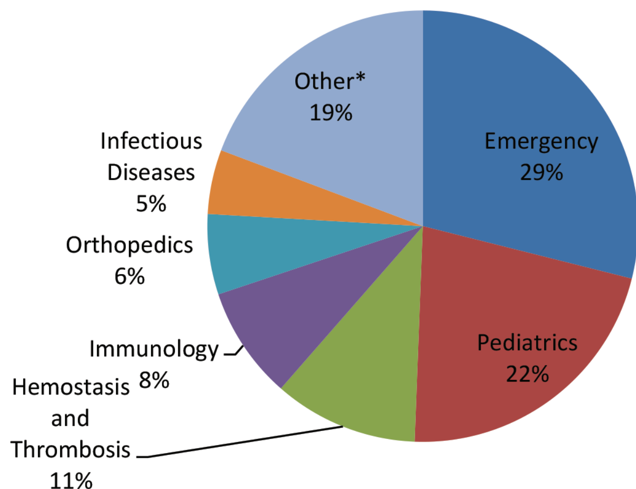

Fig. 1 - Origin of synovial fluids analyzed from January 2018 to November 2019. (*Other: Internal Medicine, Surgery, Gastroenterology, Outpatients).

Numerical results for WBC count were available for 74 of 83 samples. Results were distributed in a broad range: 63 to 128000 cells/µL, with a median value of 12825 WBC/µL.
Eight samples (10.8%) were within physiological range (< 200 WBC/µL), while 55 samples (75.1%) were in pathological range (>2000/µL). (Table 1). 11 samples (14.9%) showed results between normal and inflammatory levels (“grey zone”).
Results of WBC count and morphological evaluation obtained by microscopy were confirmed by XN 9000 Body Fluid mode (WBC-BF and PMN parameters) (Figure 2).

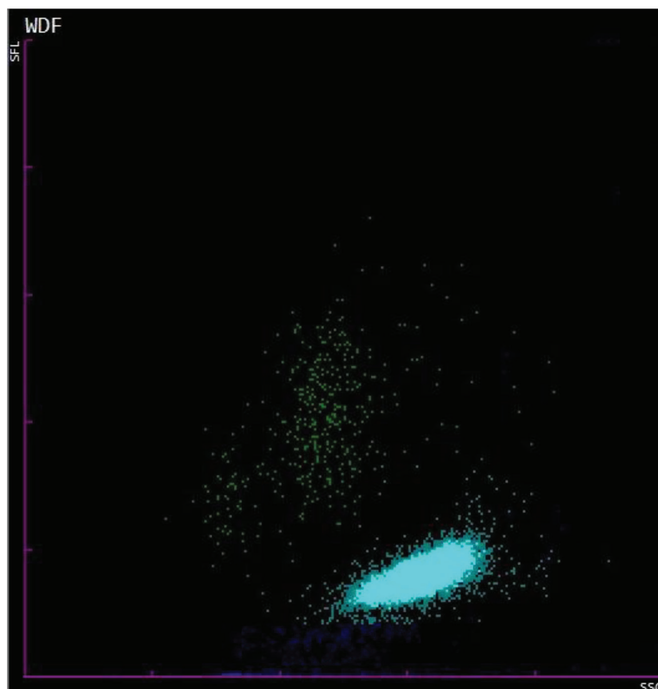

Fig. 2 - Example of WBC scattergram in a synovial fluid (XN 9000 Body Fluid mode) [light blue: polymorphonuclear cells (PMN), green: mononuclear cells (MN; lymphocytes, monocytes, synoviocytes and macrophages)].

Conclusions

Even if synovial fluid cell count could appear a very simple laboratory test, some important issues should be considered: first, a correct pre-analytical phase is mandatory for the reliability of the results. Indeed, the lack of pre-treatment with hyaluronidase or N-acetylcysteine could produce an inaccurate result and hinder the morphological evaluation of samples, due to the high viscosity of the sample. This is true both for manual count and for automated analysis. Previous studies[4] showed that XN-body fluid mode could be a reliable alternative to microscopy, however it requires pre-treatment so as manual method, in order to avoid problems in sample suction.
The lack of standardization of pre-analytical phase and the lack of diagnostic and clinical thresholds universally accepted (mostly for PJIs) are the most important issues about synovial fluid analysis: in the future the use of standardized protocols will be indispensable for the improvement of the clinical usefulness of this test.
About the experience of our laboratory, we could note that samples were sent mostly from Emergency depart-

ment, then from Pediatrics, probably due to the presence of a population of children affected by rheumatic diseases and connective tissue diseases treated in our specialized pediatric unit.

Most of results were in the pathological range, so we could affirm that requests by clinicians were appropriate.

Synovial fluid analysis plays a key role in the differential diagnosis of joint diseases, but nowadays this test is still affected by some pre-analytical factors and by the heterogeneity of clinical cut-offs proposed. The use of new automated cell counter could be a valid aid for an accurate result, but correct pre-analytical and analytical phases are anyway essential.

Table 1

	Number	%
<200 Normal	8	10.8%
>2000 Inflammation	45	61.6%
>50000 Septic arthritis	10	13.5

Tab. 1: WBC count results (cells/µL) of synovial fluids analyzed from January 2018 to November 2019.

References

1. Goldring RS, Firestein GS, Budd RC. Biology of the normal joint- Kelley's textbook of Rheumatology - 9e Elseviers, Saunders ed 2010:1533-1553.
2. Swan A, Amer H, Dieppe P . The value of synovial fluid assays in the diagnosis of joint disease: a literature survey Ann Rheum Dis 2002;61:493–498.
3. Cohen AS, Goldenberg D. Synovial fluid. In: Laboratory diagnostic procedures in the rheumatic diseases. 3rd ed. New York: Grune and Stratton Cohen AS ed., 1985.
4. Seghezzi M, Buoro S, Manenti B et al. Optimization of cellular analysis of synovial fluids byoptical microscopy and automated count using the Sysmex XN body fluid mode. Clin Chim Acta. 2016; 462: 41-48.
5. Hassan AS, Rao A, Manadan AM et al. Peripheral bacterial septic arthritis. J Clin Rheumatol. 2017; 23(8):435-442.
6. Ting NT, Della Valle CJ. Diagnosis of periprosthetic joint infection-an algorithm-based approach. J Arthroplasty. 2017; 32(7): 2047-2050.
7. Kjeldsberg's C, In: Synovial fluid , Body Fluid Analysis ,Singapore, editor JW Hussong and CR Kjeldsberg 2015; 7:139-140.
8. Kerolus G, Clayburne G, Schumacher HR Jr et al. Is it mandatory to examine synovial fluids promptly after arthrocentesis? Arthritis Rheum. 1989; 32 (3): 271-278.
9. Ottink KD, Strahm C, Muller-Kobold A et al. Factors to consider when assessing the diagnostic accuracy of synovial leukocyte count in periprosthetic joint infection. J Bone Jt Infect. 2019; 4(4): 167-173.