Coll. Antropol. **34** (2010) 1: 145–152
Original scientific paper

# The Importance of Urgent Cytological Examination of Synovial Fluids in Differentiation Inflammatory and Non-inflammatory Joint Diseases

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**A B S T R A C T**

*The aim of this study was to imply the possibilities of the urgent cytological examination of synovial fluids in differential diagnosis of arthropathies and to motivate the clinicians to use this method. It gave valuable information particularly with respect to differentiate the inflammatory and non-inflammatory joint diseases. This study included 115 synovial fluids obtained by fine needle aspiration (FNA) of the swollen knee from the patients in the period between 2003 and 2008. At our department the urgent cytological examination of the synovial fluids consisted of macroscopic analysis that includes volume, colour, clarity, viscosity and mucin clot test, native microscopic analysis for crystals and tissue fragments, counting the total nucleated cell count and semiquantitative microscopic analysis for neutrophil granulocyte percentage on the slides stained with Hemacolor rapid staining. All cytological analyses were done within one hour since FNA. According to our results the clarity, viscosity, mucin clot test, the total nucleated cell count and the neutrophil granulocyte percentage enabled distinction between inflammatory and non-inflammatory diseases with statistically significant difference at the 0.01 level but we could not differentiate these two groups of illnesses according to volume and colour. In inflammation the total nucleated cell count and the neutrophil granulocyte percentage was greater than in non-inflammation, the clarity was only translucent and opaque, the viscosity was low and the mucin clot test was negative. In non-inflammatory diseases the clarity varied from transparent to opaque, the total nucleated cell count and the neutrophil granulocyte percentage was smaller than in inflammatory diseases, the viscosity was high and consequently the mucin test was highly positive in all samples. Crystals were detected in only 12 samples of synovial fluids, mostly in inflammation and they were all monosodium urate (MUS) so we could diagnose gout. We could conclude that the urgent cytological analysis of the synovial fluid is a very useful, simple and reliable basic diagnostic screening test in differentiation inflammatory and non-inflammatory joint diseases and we recommended using it as the initial test in the diagnostic procedure of these illnesses using our protocol.*

***Key words:*** *cytology, synovial fluid, macroscopic analysis, nucleated cell count, neutrophil granulocyte percentage, crystals, joint disease*

## Introduction

Synovial fluid analysis is one of the few laboratory tests that is exclusively used for the diagnosis or assessment of musculoskeletal diseases. Ropes and Bauer were among the first to point out that differences in the appearance and cell content of abnormal synovial fluid could distinguish inflammatory and non-inflammatory

forms of arhtritis[1]. Hollander et al. recommended the routine use of macroscopic examination, cell counts, microbiology and biochemical tests of synovial fluid to differentiate the various forms of arthritis introducing the term »synovianalysis«[2].

Cytological analysis of the synovial fluid is one of the methods that give valuable information particularly with respect to the differential diagnosis of arthropathies. It consists of the macroscopic analysis (volume, colour, clarity, viscosity, mucin clot test) and microscopic qualitative and quantitative analysis (total and differential cell count, »wet prep« and cytospin smears microscopic analysis) and has diagnostic and prognostic value of rheumatic diseases[3–8]. It should be included in the initial evaluation of the most arthritic conditions that generate effusions since it enables the distinction between inflammatory and non-inflammatory illnesses, determination of the duration period of the illness and the intensity of the inflammation, as well as the control of the efficiency of the therapy[9].

However, in Croatia, as in many other countries, synovial fluid analysis (with the exception of microbiological assays) has not been established as a routine procedure within hospital cytology departments. Therefore we wanted to indicate the possibilities of the cytological examination of synovial fluids that is done immediately after bringing the fluid in the laboratory which includes the macroscopic analysis, assessment of the total number of nucleated cells and neutrophil percentage and microscopic identification of crystals and tissue fragments. The aim of this paper is to show the importance of these parts of urgent cytological analyses of the synovial fluids in distinguishing inflammatory and non-inflammatory diseases in the early stages.

## Materials and Methods

This is a retrospective study which included synovial fluids from a total of 115 patients suffering of swollen knee. The samples were processed at the Department of Clinical Cytology and Cytometry, University Hospital Dubrava in Zagreb, Croatia, during the period from 2003 till 2008.

Synovial fluids were obtained by fine needle aspiration (FNA) of knee performed by rheumatologists or orthopaedic surgeons or by cytologists with or without ultrasound guidance under sterile condition. FNA was performed using a 20-gauge needle due to viscosity of the fluid and 20-mL syringe. The fluid should be in anticoagulant because it has a tendency to clot and the best one is lithium heparin. It was immediately taken for analysis to prevent the destruction of the cells and creating the deposit, best within 2 hours since the moment of FNA because it cannot be fixed[10]. If the sample is kept in the refrigerator it could be analysed within 48 hours since FNA, preferably within the first 24 hours[5]. At our department synovial fluid samples were examined in four steps within one hour since FNA.

### *Macroscopic analysis*

Macroscopic analysis of synovial fluids includes volume, colour, clarity, viscosity and mucin clot test. The volume was expressed in millilitres. A part of the synovial fluid was injected into a test-tube and colour and clarity were determined (Figure 1). The viscosity test may be done by putting a drop of the synovial fluid between a thumb and index finger and then by measuring the continuing string (Figure 2). Viscosity is normal if the string is 3 cm long, low when the string length is less than 3 cm and high when it is longer than 3 cm. Mucin clot test was done by adding and mixing the drop of synovial fluid into 5% diluted acetic acid solution allowing it to stand about one minute. Mixing synovial fluid and acetic acid leads to the formation of a white precipitate, produced by aggregation of proteins and hyaluronans. The test is positive when a firm homogeny deposit is created.

*Fig. 1. Determination of the colour and clarity of the synovial fluid – the sample is yellow and slightly translucent.*

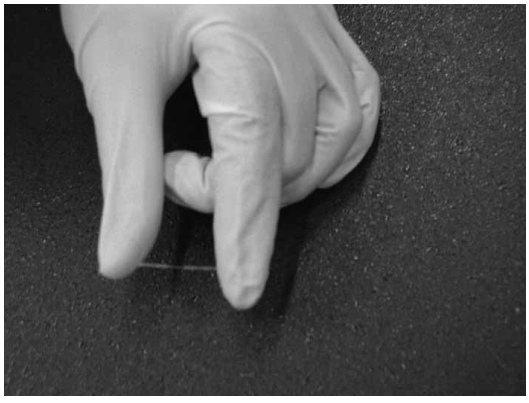

*Fig. 2. Viscosity test – it is low (the string is shorter than 3 cm).*

### *The total number of nucleated cells*

A measured aliquot of synovial fluid was diluted in a 0.01% solution of methyl violet and the total number of nucleated cells *per* $mm^3$ of fluid was established by counting supravital stained nucleated cells in a Bürker-Türk counting chamber by two persons independently of one another. Fluids with total nucleated cell count greater than $0.4x10^9$/l were diluted with normal saline to an optimal concentration. If there were a large number of the cells it was a case of automated counting as well.

### *Native microscopic analysis*

Synovial fluid often contains small particles that can be recognized with the naked eye. During the preparation of the »wet prep« it is necessary to pipette as many of those visible particles as possible and a few drops of fluid were spread onto a microscope slide. The drops were gently squeezed flat beneath a cover slip and viewed unstained with a conventional microscope. The preparations were examined for crystals, cartilage and tissue fragments by »wet preparation« microscopic analysis also by two independent persons, as well as the total cell count. The crystals can be free or phagocytes in leucocytes. The most common were pathogenic crystals monosodium urate (MSU) typically needle shaped usually 5–30 μm long (Figure 3) as a sign of gout[11].

### *The neutrophil granulocyte percentage*

The rest of synovial fluid was used for preparation of cytospin sediments. Depending on cell concentration we took 400–600 μL for cytocentrifugation and 4 slides *per* one sample were prepared and one of them was stained with Hemacolor rapid staining while the others were left for standard staining and additional cytological analysis. We analysed rapid stained smears for neutrophil granulocyte percentage using the light microscope (Figure 4).

### *Statistical analysis*

Distributions of the tested features were shown both in tables and graphically, in which descriptive statistical methods were used. The methods used in the statistical significance testing of the relationship among quantitative features were the $\chi^2$-test, the independent sample t-test and the Welch's t-test for independent samples at the 0.05 significance level. The $\chi^2$-test was used to test the difference between inflammatory and non-inflammatory diseases in sex, clarity, viscosity and mucin clot test while the independent sample t-test was used to test the difference between inflammatory and non-inflammatory diseases in volume. The Welch's t-test was used to test the difference between inflammatory and non-inflammatory diseases in the total cell count and neutrophil granulocyte percentage because equal population variances were not assumed (tested with the Levene's test).

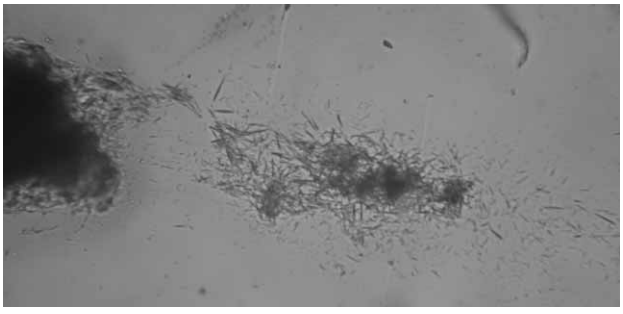

*Fig. 3. »Wet preparation« of pathogenic crystals monosodium urate (MUS) typically needle shaped (sign of gout).*

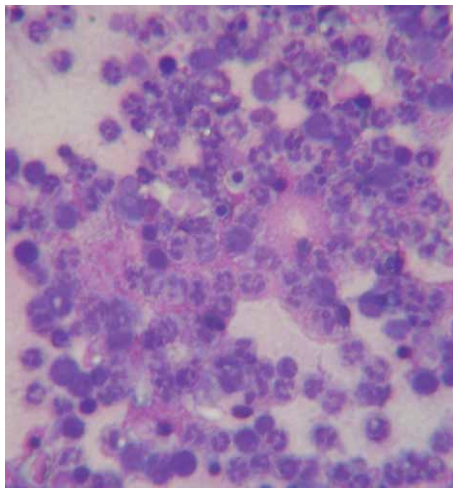

*Fig. 4. Few mononuclear cells and lots of neutrophil granulocytes (400x, Hemacolor rapid staining).*

## Results

This paper included samples of synovial fluids from 115 patients, 73 (63.5%) females and 42 (36.5%) males with a median age of 58 years (11–90) (Table 1). The pathological changes were divided into two groups: inflammatory rheumatic diseases and non-inflammatory diseases (degenerative illnesses and traumas). Most of the patients, 81 out of 115 (70.4%) were diagnosed with inflammatory diseases (Table 1). Men more often than women suffered from inflammatory diseases with statistically significant difference at the 0.01 level (p=0.006, Table 2).

As Figure 5 shows, the patients were divided into 5 age groups. The first group consisted of patients under

**TABLE 1**
CLINICAL FEATURES IN PATIENTS WITH ARTHROPATIES

| Variable | | Number | % |
|---|---|---|---|
| Age (years; median (range)) | | 58 (11–90) | |
| Sex | Male | 42 | 36.5 |
| | Female | 73 | 63.5 |
| Disease | Inflammatory | 81 | 70.4 |
| | Non-inflammatory | 34 | 29.6 |

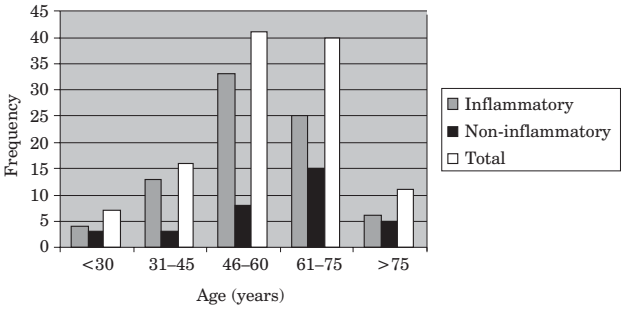


*Fig. 5. Distribution of inflammatory and non-inflammatory disases according to age.*

**TABLE 2**
DISTRIBUTION OF INFLAMMATORY AND NON-INFLAMMATORY DISEASES ACCORDING TO SEX

| Disease | Male | | Female | |
|---|---|---|---|---|
| | Number | % | Number | % |
| Inflammatory | 36 | 85.7 | 45 | 61.6 |
| Non-inflammatory | 6 | 14.3 | 28 | 38.4 |
| Total | 42 | 100.0 | 73 | 100.0 |

$\chi^2$-test statistically significant (p=0.006)

**TABLE 3**
THE VOLUME OF SYNOVIAL FLUID IN INFLAMMATORY AND NON-INFLAMMATORY DISEASES

| Disease | Volume (mL) | | |
|---|---|---|---|
| | Minimum | Maximum | $\overline{X}$±SD |
| Inflammatory | 0.1 | 25.0 | 5.73±4.09 |
| Non-inflammatory | 0.5 | 14.0 | 4.25±3.31 |

$\overline{X}$ – Mean, SD – standard deviation, t-test statistically non-significant (p=0.07)

the age of 30, the second group of patients between 31 and 45, the third group between 46 and 60, the fourth group between 61 and 75 and the last group consisted of patients over 75 years of age. In all the groups inflammatory diseases occurred more often than the non-inflammatory. It is evident that the number of patients both with inflammatory and non-inflammatory diseases grows as they age.

In inflammatory diseases the volume of the synovial fluid was greater than in the non-inflammatory diseases: mean was 5.73 mL in relation to 4.25 mL but the difference was not statistically significant (p=0.07, Table 3).

The most common colour of the synovial fluid was yellow detected in 81 out of 115 samples (70.4%) and also within both groups: inflammatory (70.4%) and non-inflammatory diseases (70.6%). Following was bloody colour with frequency of 14 (12.2%) samples. Yellow-white, yellow-green and grey colour was detected only in synovial fluids due to inflammatory diseases (Table 4).

In patients with inflammatory diseases synovial fluids were opaque (61.7%) or translucent (38.3%). In non-inflammatory diseases the synovial fluids were various: from the transparent and slightly translucent to the most common – translucent (44.1%) and opaque. The transparent and slightly translucent fluids were detected only in non-inflammatory diseases. There was statistically significant difference between both disease types according to clarity at the 0.01 level (Table 5).

**TABLE 5**
DISTRIBUTION OF CLARITY OF SYNOVIAL FLUID ACCORDING TO INFLAMMATORY AND NON-INFLAMMATORY DISEASES

| Clarity | Inflammatory disease | | Non-inflammatory disease | | Total number |
|---|---|---|---|---|---|
| | Number | % | Number | % | |
| Transparent | 0 | 0 | 6 | 17.6 | 6 |
| Slightly translucent | 0 | 0 | 10 | 29.4 | 10 |
| Translucent | 31 | 38.3 | 15 | 44.2 | 46 |
| Opaque | 50 | 61.7 | 3 | 8.8 | 53 |
| Total | 81 | 100.0 | 34 | 100.0 | 115 |

$\chi^2$-test statistically significant (p<0.01)

**TABLE 4**
DISTRIBUTION OF COLOUR OF SYNOVIAL FLUID ACCORDING TO INFLAMMATORY AND NON-INFLAMMATORY DISEASES

| Colour | Inflammatory disease | | Non-inflammatory disease | | Total | |
|---|---|---|---|---|---|---|
| | Number | % | Number | % | Number | % |
| Yellow | 57 | 70.4 | 24 | 70.6 | 81 | 70.4 |
| Jade yellow | 2 | 2.5 | 2 | 5.9 | 4 | 3.5 |
| Yellow-white | 8 | 9.8 | 0 | 0.0 | 8 | 7.0 |
| Yellow-green | 6 | 7.4 | 0 | 0.0 | 6 | 5.2 |
| Grey | 2 | 2.5 | 0 | 0.0 | 2 | 1.7 |
| Bloody | 6 | 7.4 | 8 | 23.5 | 14 | 12.2 |
| Total | 81 | 100.0 | 34 | 100.0 | 115 | 100.0 |

**TABLE 6**
DISTRIBUTION OF VISCOSITY OF SYNOVIAL FLUID ACCORDING TO INFLAMMATORY AND NON-INFLAMMATORY DISEASES

| Viscosity (string length) | Inflammatory disease | | Non-inflammatory disease | | Total number |
|---|---|---|---|---|---|
| | Number | % | Number | % | |
| Low (<3 cm) | 72 | 88.9 | 4 | 11.8 | 76 |
| High (>3 cm) | 9 | 11.1 | 30 | 88.2 | 39 |
| Total | 81 | 100.0 | 34 | 100.0 | 115 |

$\chi^2$-test statistically significant ($p<0.01$)

**TABLE 7**
DISTRIBUTION OF MUCIN CLOT TEST RESULTS OF SYNOVIAL FLUID ACCORDING TO INFLAMMATORY AND NON-INFLAMMATORY DISEASES

| Mucin clot test | Inflammatory disease | | Non-inflammatory disease | | Total number |
|---|---|---|---|---|---|
| | Number | % | Number | % | |
| Negative | 73 | 90.1 | 0 | 0 | 73 |
| Positive | 8 | 9.9 | 34 | 100.0 | 42 |
| Total | 81 | 100.0 | 34 | 100.0 | 115 |

$\chi^2$-test statistically significant ($p<0.01$)

The viscosity of the synovial fluids was low in the most of the patients with inflammatory diseases, in 72 out of 81 and it was high in the most of the patients with non-inflammatory diseases, in 30 out of 34. There was statistically significant difference between viscosity in inflammatory and non-inflammatory diseases at the 0.01 level (Table 6).

All the synovial fluids in the non-inflammatory diseases had a firm deposit of mucin (clot test was highly positive), while in the inflammatory diseases mucin clot test was more often negative than positive, 73 in relation to 8. It is obvious that there was statistically significant difference between these two groups according to mucin clot test results at the level 0.01 (Table 7).

The total cell count expressed in mean was 30353/mm$^3$ in inflammatory diseases that is 71 times higher than in non-inflammatory diseases in which the mean was 428/mm$^3$. It is not surprisingly that there was statistically significant difference between inflammation and non-inflammation at the level 0.01 (Table 8). The neutrophil granulocyte percentage expressed in mean was 3 times higher in inflammation (63.2) than in non-inflammation (19) with statistically significant difference at the level 0.01 (Table 9).

**TABLE 8**
THE TOTAL NUCLEATED CELL COUNT *PER* MM$^3$ OF SYNOVIAL FLUID IN INFLAMMATORY AND NON-INFLAMMATORY DISEASES

| Disease | Total cell count *per* mm$^3$ | | |
|---|---|---|---|
| | Minimum | Maximum | $\overline{X}$±SD |
| Inflammatory | 2000 | 220000 | 30353±43732 |
| Non-inflammatory | 201 | 1276 | 428±345 |

$\overline{X}$ – Mean, SD – standard deviation, Welch's t-test statistically significant ($p<0.01$)

**TABLE 9**
THE NEUTROPHIL GRANULOCYTE PERCENTAGE IN SYNOVIAL FLUID IN INFLAMMATORY AND NON-INFLAMMATORY DISEASES

| Disease | Neutrophil granulocyte percentage | | |
|---|---|---|---|
| | Minimum | Maximum | $\overline{X}$±SD |
| Inflammatory | 20 | 97 | 63.21±20.28 |
| Non-inflammatory | 10 | 34 | 19.00±9.17 |

$\overline{X}$ – Mean, SD – standard deviation, Welch's t-test statistically significant ($p<0.01$)

The crystals were detected in only 12 out of 115 synovial fluids by the native microscopic analysis, mostly in patients with acute inflammation – in 11 of them. All crystals were MSU. The fragments of the cartilage were detected in only 3 patients, 2 with inflammation and 1 with non-inflammation (Table 10).

## Discussion and Conclusion

The normal synovial fluid is a dialysate of plasma with liquid and cellular elements. It is clear, transparent, straw-like yellow, avascular, hypocelular (10–200 mononuclear cells *per* mm$^3$) and present in small quantities

**TABLE 10**
DISTRIBUTION OF CRYSTALS AND FRAGMENTS IN SYNOVIAL FLUID ACCORDING TO INFLAMMATORY AND NON-INFLAMMATORY DISEASES

| Crystals and fragments | Inflammatory disease | | Non-inflammatory disease | | Total number |
|---|---|---|---|---|---|
| | Number | % | Number | % | |
| No crystals | 68 | 84.0 | 32 | 94.2 | 100 |
| Crystals (MSU) | 11 | 13.5 | 1 | 2.9 | 12 |
| Cartilage fragments | 2 | 2.5 | 1 | 2.9 | 3 |
| Total | 81 | 100.0 | 34 | 100.0 | 115 |

(0.1–3 mL). It does not contain fibrinogen and therefore it does not coagulate, but it has a hyaluronic acid produced by type B synovial cells. That fact results in high viscosity and highly positive mucin clot test[12].

The synovial fluid examination is excellent diagnostic screening test. Cytological analysis is non-invasive method that can lead to diagnosis and gives prognostic data but unfortunately is not used often enough in the diagnostic procedure. Swan et al. identified 6556 papers for the period 1980–2001 using the key word »synovial fluid« and when the search was combined with the key word »cytology«, only 33 papers were identified[13]. Hasselbacher has shown that the frequency of synovial fluid analysis in 42 hospitals from New Hampshire and Vermont was low (mean was 3.9 *per* month)[14]. Amer et al. recommended that there is an urgent need for guidelines, standardization and education about the use of synovial fluid assays in the United Kingdom. They surveyed 535 rheumatologists and orthopaedists about the analysis of the synovial fluid and got feedback from 311 of them who regularly used only microbiological test and polarized microscopy for the diagnosis of acute arhtritis[15]. Similar situation is present in Croatia. The most probable reason is that too few laboratories offer investigation and too few clinicians demand it.

According to our results, in inflammatory diseases the volume of synovial fluid was greater than in non-inflammatory diseases and the colour of the synovial fluid varied from yellow through yellow-green to bloody. The total cell count and neutrophil granulocyte percentage was greater as the illness was more acute and consequently the fluids were translucent to opaque. The viscosity was very low and consequently the mucin clot test was negative. With non-inflammatory diseases (degenerative illnesses and injuries) the amount of the synovial fluid was smaller with a smaller range and the colour was yellow, jade yellow or bloody. The clarity varied from transparent to opaque, the total cell count and neutrophil granulocyte percentage was small, the viscosity was high and consequently the mucin test was highly positive in all samples.

The volume of the synovial fluids was larger in inflammatory than in non-inflammatory diseases. That is because the permeability of the synovial cells that discharge fluid is increased, and also because of the increased number of the cells due to the inflammatory reaction or irritation by the fragments of the cartilage or the crystals. But, we couldn't differentiate inflammatory from non-inflammatory diseases according to volume. The colour of the synovial fluid did not enable the distinction of the inflammatory from the non-inflammatory diseases, too because many of the different illnesses had the same colour that is evident from the Table 4. However, there are exceptions such as septic arthritis with grey colour due to the bacterial chromogens or gout with milky white fluid. That, along with the other parameters, can indicate not only the inflammation but also the specific inflammatory process[4,12,16].

The clarity, viscosity, mucin clot test, the total cell count and neutrophil granulocyte percentage showed statistically significant difference at the 0.01 level between inflammatory and non-inflammatory diseases and therefore enabled distinction between these two groups of diseases as it is shown in Tables 5–9.

The clarity of the synovial fluids depends on the number of cells and therefore the synovial fluid is opaque when a large number of cells are present as well as crystals. Opposite, the synovial fluid is transparent and slightly translucent when a small number of cells are present. It also depends on the bleeding due to trauma and on the amount of tissue fragments and cartilage, which is usually found in degenerative rheumatic diseases[12].

The viscosity of the joint fluids is determined by the amount of the hialuronic acid and other mucopolisaccharides. That is important because it enables sliding of the joint surfaces during walking. The viscosity is decreased during inflammation due to enzymes digestion and depolimerization of the mucopolisaccharides and therefore it is possible, based on the analysis, to distinguish inflammation from degenerative illnesses[3,4].

The mucin deposit was firm in all the samples of the non-inflammatory changed synovial fluids, while it was loose in most samples of inflammatory changed synovial fluids and hence enabled us to differentiate inflammation from non-inflammation when the mucin clot test was negative. It is necessary to be careful with haemorrhage because it prevents mucin clot formation[10,12].

The cell count *per* $mm^3$ together with the neutrophil granulocyte percentage enables not only to distinguish inflammatory from non-inflammatory diseases, but also to determine inflammation stage – if it is acute or subacute, whether it is septic arthritis or not. Trampuz et al. had sensitivity of 94% and specificity of 88% when determining total cell count in 133 patients and significantly higher count in patients with prothetic joint infection than in those with aseptic failure[17]. Our patients with inflammations had a cell count 71 times higher than those with non-inflammation diseases which coincides with the results of other authors[3,10,18]. The greater the cell count, the more urgent it is to start a treatment since it can be a first sign of a septic arthritis[19]. Davies et al. pointed out that the cell count has the diagnostic and prognostic value in the patients suffering of rheumatoid arthritis: if the cell count is smaller, the prognosis is better[8]. Despite the great importance of determining the cell count while diagnosing arthritis, there is still not a perfect measurement established to prevent errors. Jonge et al. implied that with small number of cells, it is more precise to count the cells manually, but when a large amount of cells is present automated leukocyte counting in synovial fluid is more precise than manual counting[20]. It is important that the cells are counted by an experienced person. Salinas et al. indicated that in such a case usually the manual number of cells overlaps with the one acquired by the automated counting[21]. Therefore, in cases of a large number of cells we have also counted the cells by both methods and the manual counting was always con-

ducted by two persons, independently of one another due to subjective analysis, in order to avoid errors, as it was done by other authors[14–15,21]. The neutrophil granulocytes are predominant cells in inflammatory arthropathies as a consequence of specific traffic into the synovial fluid and in intra-articular haemorrhage because they are the most abundant nucleated cell in blood. Therefore in these conditions the most common neutrophil granulocytes percentage is 60–80% of all nucleated cells[12]. Trampuz et al. had sensitivity of 97% and specificity of 98% when determining neutrophil granulocyte percentage[17]. Our patients with inflammation had neutrophil percentage three times higher than those with non-inflammation that is comparable with the results of other authors[12,17,19]. The difference between us with others is only in method because we differentiate cells immediately on rapid stained smears and the others do it on standard stained cytospin smears. If the neutrophil percentage is more than 90–95%, then septic arthritis should be taken in consideration even when organisms cannot be identified[12,19,22] although Mathews et al. have opposite opinion[23].

By native analysis of the joint fluid it can be determined whether crystals are present or absent and then identified the crystal type: (MSU) with gout, calcium pyrophosphate dehydrates (CPPD) with deposit disease, calcium hydroxyapatite, lipid liquid with acute and chronic arthritis and cholesterol with chronic rheumatoid effussions[24]. Identification of crystals in the synovial fluid is mandatory for the diagnosis of microcrystal deposition arhtropathy but it depends on subjective analysis. That is why it is required for the experienced person or persons to test the same specimen independently of each other as an internal control, as we did in our department. In our specimens, we have identified crystals of MSU only in 12 synovial fluids so with the other parameters we were able to set a diagnosis of the acute gout in 11 patients and chronic gout in one patient. Lumbreras et al. analysed 194 synovial fluid samples and detected 55 with CPPD crystal and 43 with MSU crystals with sensitivity of 95.9% and specificity of 86.5% for crystal detection[25]. For identification of MSU sensitivity was 95.5% and specificity 97.2% done with an ordinary light microscope[25]. Since it is important to detect MSU crystals as soon as possible and thus prevent the development of the illness, some prefer polarised light microscope to diagnose an attack of gout or pseudogout[26]. The others intercede that, the basic calcium phosphate crystals which is unique to osteoarthritis, would be detected more precisely with scanning electron microscopy[27]. Yuan et al. have noticed that sometimes in patient with gout a reanalysis of the synovial fluid for urate crystals on the same sample a day later can be positive even though only a day earlier the test was negative by light and polarised microscope in 7 out of 30 samples (24%)[28]. Although it is recommended that synovial fluid should be analysed preferably within a few hours since arthrocentesis because a significant fall in the number of CPPD crystals may occur, Galvez et al. pointed out that when MSU and CPPD crystals were initially detected in synovial fluid they could still be seen 24 and 72 hours later in 97%/96% (MSU) and in 100%/97% (CPPD) cases when samples were stored at 4 °C whether or not anticoagulant was used[29]. This fact is important since it is not always possible to analyse the fluid immediately and also it can be used for the purpose of the internal control.

Our results showed that the macroscopic analysis, the determination of the cell count and neutrophil granulocyte percentage as well as the microscopic native analysis of the synovial fluids enabled the differentiation of the inflammatory from the non-inflammatory joint diseases, and in some cases these procedures enabled diagnosing a specific rheumatiod illness, such as gout, by the identification of the crystals or a septic arthritis based on a cell count, colour, viscosity and mucin test even before the typical symptoms occurred. Ma et al. came to similar conclusions based on studying the differential diagnosis of acute monoarthritis with 50 year old patients[30]. Opposite to our results, some authors indicated that the macroscopic analysis of the joint fluids was sufficient to distinguish inflammatory from non-inflammatory diseases and that it was unnecessary to conduct a total cell count[16,31]. Abdullah et al. have analysed 80 synovial fluids in such a way with sensitivity of 94% and specificity of 58%[31]. Given our experience into consideration, we would not agree with that conclusion, but quite to the opposite, we think that all the analyses are necessary putting the pieces of diagnostic puzzle together.

In order for the analysis to be adequate, the experience of the engineers and the doctors that analyse the synovial fluids is needed, since the most of the tests depend upon the subjective evaluation. Therefore we would recommend that some examinations such as the cell count and crystal analysis should be performed by two or three persons independently one of another, as suggested by some other authors as well[9,11,14–15,25].

In summary, the urgent cytological analysis of the synovial fluid is a very useful, simple and reliable basic diagnostic screening test for patients with arthropathies. It can be done in every patient in every hospital that has a cytological laboratory and all the results can be completed within few hours the same day. That can promptly indicate a potential illness and improve the prognosis on one hand, and rationalise the tests, reduce the time and the cost *per* patient on the other side. Therefore we recommend that the urgent cytological analysis of the synovial fluids should be used as much as possible as the initial test in the diagnosis of joint disease using our protocol.

## Acknowledgements

This study is a part of the Research project No. JMV 198-1081874-0183 supported by the Ministry of Science, Education and Sports of the Republic of Croatia.

The authors would like to thank the members of the Department of Clinical Cytology and Cytometry, particularly the cytotechnologists Samir Bezdrob and Biserka Cvrk.

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## VAŽNOST HITNE CITOLOŠKE ANALIZE ZGLOBNIH TEKUĆINA U RAZLIKOVANJU UPALNIH I NEUPALNIH BOLESTI ZGLOBOVA

## SAŽETAK

Cilj ovog rada je ukazati na mogućnosti hitne citološke analize zglobnih tekućina u dijagnosticiranju različitih artropatija i motivirati kliničare da koriste ovu analizu prilikom obrade pacijenata s različitim reumatskim bolestima. Hitna citološka analiza omogućuje razlikovanje upalnih od neupalnih bolesti, a ponekad i postavljanje precizne dijagnoze bolesti kao npr. gihta. Ova studija uključuje 115 zglobnih tekućina dobivenih citološkom punkcijom otečenog koljena u pacijenata u razdoblju od 2003. do 2008. godine. Na našem odjelu hitna citološka analiza zglobnih tekućina se sastoji od makroskopske analize koja uključuje volumen, boju, zamućenost, viskoznost i mucin test, nativnu mikroskopsku analizu kristala i tkivnih fragmenata, od brojenja ukupnog broja stanica s jezgrom i od semikvantitativne mikroskopske analize postotka neutrofilnih granulocita svjetlosnim mikroskopom na preparatima obojenim hitnom metodom bojenja po Hemacoloru. Sve ove citološke analize se obavljaju unutar jednog sata od obavljene punkcije. S obzirom na naše rezultate zamućenost, viskoznost, mucin test, ukupni broj stanica s jezgrom i postotak neutrofilnih granulocita omogućuje razlikovanje upalnih od neupalnih reumatskih bolesti sa statistički vjerodostojnom razlikom na razini $p<0.01$ dok se na temelju analize volumena i boje to ne može učiniti. Kod upala ukupni broj stanica s jezgrom i postotak neutrofilnih granulocita je veći nego kod neupala, zglobna tekućina je samo zamućena ili mutna, viskoznost je niska, a mucin test je negativan. U neupalnim reumatskim bolestima zglobna tekućina može biti raznolika: od prozirne do mutne, ukupni broj stanica s jezgrom i postotak neutrofilnih granulocita je manji nego kod upala, viskoznost je jako velika i sukladno tome mucin test je jako pozitivan u svim uzorcima. Kristali su nativnom mikroskopskom analizom pronađeni samo u 12 sinovijalnih tekućina kod upala i svi su bili urati tako da se citološki mogla postaviti dijagnoza gihta kod tih pacijenata. Možemo zaključiti da je hitna citološka analiza zglobnih tekućina vrlo korisna, jednostavna i predstavlja bazični dijagnostički skrining u razlikovanju upalnih od neupalnih reumatoloških bolesti te zbog toga preporučamo da se koristi kao početna pretraga u dijagnostičkoj proceduri artropatija prema našem protokolu.