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Role of Cell Count of Synovial Fluid in Diagnosis of Joint Disease Compared to Gross Analysis

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Abstract

Joint effusion is a common clinical problem throughout the world. Synovial fluid analysis is one of the most important diagnostic tests to differentiate various inflammatory and non-inflammatory joint diseases. Gross examination and cell count of synovial fluid are two important test modalities. A comparison of performances of the tests would enable us to emphasize on the diagnostic procedures for better outcomes. A total of 105 cases were included in this study, performances of the tests such as accuracy, sensitivity, specificity, positive and negative predictive values of each test modality were calculated for comparison. Joint effusions were diagnosed as 19 rheumatoid arthritis, 12 non-inflammatory, 15 inflammatory, 14 infective, 7 tubercular, 4 traumatic, 6 osteoarthritis, 5 crystal induced arthritis, 6 non-specific arthritis and 8 normal cases. Accuracy, sensitivity, specificity, PPV and NPV of cell count were 76.19%, 72.72%, 94.11%, 98.46% and 40% respectively and accuracy, sensitivity, specificity, PPV and NPV of gross examination were 71.42%, 68.18%, 88.23%, 96.77% and 34.88% respectively. The individual parameters of synovial fluid study overlaps among each other test modalities and a combination of test procedures would yield better performances for diagnosis.

Keywords: Synovial fluid, gross analysis, Cell count, Joint effusion.

INTRODUCTION

Synovial fluid analysis has been recommended as an important aid to diagnosis and management of patients with joint diseases and it is an extremely valuable procedure in making rapid and accurate diagnosis for many types like various inflammatory, non-inflammatory, infective and crystal induced arthritis [1, 2]. In 1953, Ropes MD and Bauer W introduced cell count examination of SF for differentiation between inflammatory and non-inflammatory joint diseases [3]. The American Rheumatologic Association recommended a guideline for discrimination among inflammatory, non-inflammatory and infectious diseases by WBC count in joint fluid [4]. The test modalities such as gross analysis, cell count, polarised light microscopy, microscopy with culture and gram stain have important role in diagnosis of joint effusion, [5] and few investigators preferred gross analysis to cell count as its interpretation is not conducive in diagnosis of non-inflammatory effusion [6, 7]. In interpreting gross analysis, there is considerable overlap of findings, inter observer variation and physician bias, on the other hand, Nowadays automated techniques and other laboratory procedures have improved the outcome of cell count [8]. It is important to evaluate the performances for comparison between outcomes of gross analysis and cell count to assess their reliability.

MATERIALS AND METHODS

A cross-sectional prospective study was carried out including 105 consecutive synovial fluid samples collected from the patients attending Khulna City Medical College Hospital with joint diseases during the period from July 2021 to June 2022. After obtaining the informed consent of the patients and considering the inclusion and exclusion criteria, all the participants were admitted into the hospital and each patient was assigned a unique number and all demographic particulars and clinical information's were recorded in a prescribed form. After admission, bedside sample of at least 2 ml. of synovial fluid were collected in an anticoagulated glass container aseptically not only to prevent transmission of infection but also to ensure

accurate fluid analysis and after collection by a physician with intimate knowledge of the involved joint, the fluid was examined by clinical staffs and findings were recorded. Immediately after examination, the labelled sample was sent to the laboratory within one hour for subsequent laboratory procedures and if any delay, the specimen was stored in a refrigerator at 4^0 C.

Aim of the present study was to compare the diagnostic accuracy, specificity, sensitivity, positive and negative predictive values of gross examination of synovial fluid with that of cell count examination. A questionnaire for gross analysis was followed and interpretation was normal and abnormal determined by agreement of bedside examiners (Table-1).

Table-1: Questionnaire for gross examination of synovial fluid [7].

1. Clarity (Newsprint test)

Transparent ☐ Translucent ☐ Opaque ☐

2. Viscosity (String test)

String only ☐ String and drop ☐ Drops only ☐

3. Colour

Colourless/straw coloured ☐

Yellow/green ☐

White/green/brown ☐

Blood stained ☐

Haemorrhagic ☐

Synovial fluid parameters are non-specific and results overlap among themselves except Gram stain, Culture and Crystal examination [8]. For comparison of the result of gross examination following the questionnaire with the result of cell count recommended by American rheumatologic association, the gross examination results were classified into normal or negative and abnormal or positive groups, and its diagnostic efficiency were verified by correlating with clinical diagnoses [9]. At the same time, diagnostic efficiency of cell count was also verified by correlating with clinical diagnosis. To compare the performances of both test modalities, cell count was done as total count and differential count. Total count was done using Neubauer's counting chamber with WBC pipette, synovial fluid was drawn up to 0.5 mark and diluted up to "11" mark with normal saline, Turk fluid was also used for dilution in cases of haemorrhagic aspirates. The differential count was done by Leishman's stain of the dried smear prepared from deposits of centrifugation. Total count of <200/c.mm and differential count of Neutrophils of < 75% were considered normal or negative and total count of >200/c.mm were classified as abnormal or positive [10]. A final diagnosis was established by evaluation of the test results and then tabulated. All the data obtained from observations were recorded and summarised and presented in charts and tables. Diagnostic accuracy, sensitivity, specificity and predictive values of gross examination and that of cell count were calculated with consideration of clinical diagnosis as "Gold standard" for comparison. Statistical analysis was performed by using a computer generated software "SPSS" 'Statistical package for the social sciences (SPSS Inc; Chicago, IL, USA)'.

RESULTS

A total of 105 samples of synovial fluid from participants having joint diseases were included in this study. The range of age of the participants was from 9 years to 82 years with median of 56, and 67% of the patients were male and 33% were female. All the cases were categorized on the basis of performances of gross examination and cell count results correlating to their diagnoses after clinical assessment shown in table-2.

Table-2: Performances of the test procedures correlating to diagnoses, (n=105)

No. of cases in Different categories	Gross examination Result(Positive/Negative	Cell count Result (Positive/Negative)	Diagnosis
12	Positive	Positive	Septic arthritis
9	Positive	Positive	Non-inflammatory
11	Negative	Negative	Rheumatoid arthritis
7	Negative	Negative	Normal
5	Positive	Positive	TB

No. of cases in Different categories	Gross examination Result(Positive/Negative	Cell count Result (Positive/Negative)	Diagnosis
13	Positive	Positive	Inflammatory
5	Negative	Positive	Crystal induced
3	Positive	Positive	Osteoarthritis
3	Negative	Positive	Traumatic
6	Positive	Negative	Arthritis(Not specified)
5	positive	Positive	Rheumatoid arthritis
2	Negative	Positive	TB
2	Positive	Negative	Septic arthritis
3	Negative	Positive	Non-inflammatory
7	Negative	Negative	Non-diagnostic
2	Positive	Negative	Osteoarthritis
2	Positive	Negative	Non-diagnostic
2	Positive	Negative	Inflammatory
3	Negative	Positive	Rheumatoid arthritis
1	Negative	Negative	Osteoarthritis
1	Positive	Positive	Traumatic
1	Negative	Positive	Normal

Among the cases, 19 cases (18.1%) were diagnosed rheumatoid arthritis, 15 cases (14.3%) were inflammatory arthritis, 12 cases (11.4%) were non-inflammatory arthritis, 14 cases (13.3%) were septic arthritis, 7 cases (6.6%) were Tubercular arthritis, 9 cases (8.5%) were non-diagnostic and 8 cases (7.6%) were normal (Table-2).

Among the 19 rheumatoid arthritis cases, 11 samples showed normal findings in both the procedures, 5 cases exhibited yellowish colour with string test negative and higher cell count, and 3 samples were clear and colourless appearance but higher cell count.

In 14 cases of septic arthritis, 12 samples showed positive findings in both the test procedures, but 2 samples exhibited normal cell count and opaque appearance with white colour and string test were negative.

In 12 cases of non-inflammatory arthritis, 9 samples exhibited positive results in both gross examination and cell count and in 3 cases, cell count were significantly higher but string tests were positive with clear and colourless appearance.

In 15 inflammatory cases, 13 samples showed positive results in both gross and cell count examination, but 2 samples exhibited normal cell count and positive findings in gross examination.

In 6 cases of osteoarthris, 3 samples showed positive results in both the test procedures and 2 cases exhibited normal leucocyte count but string test negative and turbid appearance with yellow colour. 1 sample was normal in both the tests.

Among the 7 cases of tubercular arthritis, 5 samples were abnormal in appearance with yellow colour and also had higher cell count, and 2 samples showed significantly higher cell count but normal gross appearance.

In 4 traumatic cases, cell count was increased and string test were positive with translucent appearance in 3 cases whereas 1 case exhibited positive findings in both the tests.

Table-3: The findings of gross examination of synovial fluid in different diagnoses, (n=105)

Criteria		RA	SA	Infl	Non-Inf	OA	TB	Crystal Induced	Traumatic	NS-A	Non-D
Clarity	Clear(43)	14	0	0	3	1	2	5	3	0	15
	Turbid(23)	2	3	5	2	2	4	0	1	3	1
	Opaque(39)	3	11	10	7	3	1	0	0	3	1
Viscosity	Str:+ve(43)	14	0	0	3	1	2	5	3	0	15
	Str:-ve(62)	5	14	15	9	5	5	0	1	6	2
Colour	Colourless/Straw(43)	14	0	0	3	1	2	5	3	0	15
	Yellow/Brown(43)	2	9	14	7	3	2	0	1	4	1
	Brown/Green(19)	3	5	1	2	2	3	0	0	2	1
Total Count	>200/cmm(65)	8	12	13	12	3	7	5	4	0	1
Polymorphs	>75%	0	11	7	5	1	0	3	4	0	0
Lymphocytes	>75%	8	1	6	7	2	7	2	0	0	1

Str-String test, Positive-Normal, Negative-abnormal, RA-Rheumatoid Arthritis, SA-Septic Arthritis, Infl-Inflammatory Arthritis, Non-Infl: Non-Inflammatory Arthritis, OA-Osteoarthritis, TB-Tubercular Arthritis, Crystal Induced Arthritis, Traumatic Arthritis, NS-A: Non-specific Arthritis, Non-D: Non- diagnostic and normal.

In 105 SF samples, 43 samples were normal in appearance during gross examination, 23 were turbid and 39 samples were opaque, (Table-3). 43 samples had normal viscosity and 62 samples showed string test negative, 5 samples exhibited string & drops and those were considered as normal results. 43 samples were colourless or straw coloured, 43 samples were either yellow or brown, and 19 samples were either brown or green.

In microscopic examination of all the samples, 65 samples had total cell count of more than 200/c.mm (200/c.mm to 56,000/c.mm), and 40 samples had normal cell count (<200/c.mm). Among these 65 positive samples, 31 had neutrophil of >75%, and 34 samples showed lymphocytic predominance.

Table-4: Specificity, sensitivity, positive predictive value, negative predictive value and accuracy of gross examination & cell count (n=105)

Procedure	Specificity	Sensitivity	Positive Predictive value	Negative Predictive value	Accuracy
Gross examination	88.23%	68.18%	96.77%	34.88%	71.42%
Cell count	94.11%	72.72%	98.46%	40%	76.19%
Combined	82.35%	86.36%	96.2%	53.84%	85.71%

Accuracy of cell count (76.19%) was more than accuracy of gross examination (71.42%), but combined accuracy was highest (85.71%), (Table-4). Specificity, sensitivity, positive predictive value and negative predictive value of cell count were 94.11%, 72.72%, 98.46% and 40% respectively which were more than that of gross examination of 88.23%, 68.18%, 96.77% and 34.88% respectively. Specificity, sensitivity, positive and negative predictive value of the combination of test procedures were 82.35%, 86.36%, 96.2% and 53.84% respectively. Sensitivity, negative predictive value and accuracy of combination were 86.36%, 53.84% and 85.71% respectively which were more than those of individual test modalities.

DISCUSSION

The Laboratory evaluation of synovial fluid for diagnosis of patients with a joint effusion include gross analysis, cell count, PLM and microbiological assays, and other tests such as mucin clot test, glucose, protein, pH studies have been shown to be less effective, and also a literature survey revealed that only 6.1% samples identified crystals by routine examination and this has a little clinical value when infection is not suspected [6, 7]. The aim of this study was to compare the performances of gross examination to the performances of cell count for assessment of reliabilities of both the test modalities which may lead us to become enthusiastic for the test procedures. The present study revealed that the specificity, sensitivity, PPV, NPV and accuracy of cell count (94.11%, 72.72%, 98.46%, 40% and 76.19% respectively) were more than those of gross examination (88.23%, 68.18%, 96.77%, 34.88% and 71.42% respectively), as well as the sensitivity, negative predictive value and accuracy of combination of both tests were higher(86.36%, 53.84% and 85.71% respectively), (Table-4). The findings of the present study were comparable to those observed in a study performed by Garg P and Goyal V which exhibited sensitivity and specificity of gross examination of 91% and 70% respectively whereas, those of cell count were 94% and 86% respectively [9]. Sampling of synovial fluid is a direct approach for diagnosis of joint effusion or inflamed joint and its evaluation is critical to identify acute monoarthritis, septic or crystal induced arthritis and other inflammatory arthritis [11, 12]. The gross examination of synovial fluid has contributory role in diagnosis of the patient having joint diseases regarding amount, appearance, viscosity and colour and being of haemarthrosis. Transparent synovial fluid occurs in non-inflammatory conditions, turbidity increases with increase in joint inflammation, purulent fluid occurs in infections and in the present study, the findings were similar as in normal cases and non-inflammatory cases, and the fluids were clear or yellow and viscous, whereas turbidity increases with negative string test in the inflammatory conditions found in some studies [13, 14]. Synovial fluid volume was increased as the inflammatory reaction increases reported by a study in which viscosity was reduced, turbidity increased, the cell count was increased, and also the PMN ratio was increased [15]. These findings are consistent with the present study revealing that synovial fluid in conditions like septic arthritis having marked joint inflammation, is cloudy and purulent, have low viscosity with high total count of leucocytes.

The limitations of the present study were firstly, variations of inter observer agreement were not calculated statistically, secondly, considerable overlap among the findings of gross examination are evident and further development of questionnaire is essential to minimize it, thirdly, the cell count techniques of present study were not verified by automated counting method and other laboratory procedures were not followed, fourthly, the cases with effects of DMARD were not excluded from the study and finally, other test modalities such as biopsy, cytological study, cultures, staining and arthroscopy were not utilized for diagnosis of joint diseases.

A small number of samples from rheumatoid arthritis cases showed cellular changes in microscopic examination and gross examination were also had no deciding role, but studies performed by Yu MX and

Hollander with rheumatoid arthritis cases revealed that marked variation of leucocyte count from 330- 72600 cell/cmm with 9-97% of polymorphonuclear leucocytes, and another report showed the cell count were 2660-3000 cells/cmm with polymorphs of >50% [16, 17].

In examination of synovial fluid from septic arthritis cases, most of the samples were turbid, opaque, shown negative string test, yellow to brown colour and high cell count such as 12 cases showed total count more than 50,000/cmm with neutrophil predominance(>75%), and both the tests were highly specific and sensitive for diagnosis. The clinical presentation of septic arthritis may overlap on other acute arthritis cases, for this reason it is important to examine synovial fluid both grossly and microscopically [18]. In the cases of tubercular arthritis, most of the samples were straw colour and normal appearance and few cases showed high cell count of range from 2000/cmm to 10000/cmm, with lymphocytic predominance, which were similar with the findings of some studies [19, 20].

In non-inflammatory cases, a considerable number of samples showed positive result in both tests, although gross examination has an important role to discriminate between inflammatory and non-inflammatory arthritis cases, also it was evident that the sensitivity, negative predictive value and accuracy of the combination were better than the individual test modality. A literature survey stated that only microbiological test was standardised within the health service, and the synovial fluid study was excluded from routine diagnostic tests as the poor standard of fluid study and lack of quality control leading us to remain in the ignorance [21].

Further study is recommended within the specific diagnosis of the cases having joint effusion to minimize the limitations in interpretation of results of gross examination due to overlap and for standardisation of laboratory method of cell count.

CONCLUSION

The synovial fluid analysis is an important laboratory test for diagnosis and management of patients with joint diseases. The overall performance of cell count is better than that of gross examination and the combination of these two test procedures is preferable because of higher sensitivity, negative predictive value and accuracy.

Ethical Clearance

The research protocol for this study and informed consent form were reviewed and approved by the ethical committee of Khulna City Medical College. The written informed consent was then obtained from each participant and a unique identification number was assigned and all records were kept in a secured room to ensure confidentiality.

Conflict of Interest: None.
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