

SYNOVIAL FLUID ADENOSINE DEAMINASE ACTIVITY TO DIAGNOSE TUBERCULOUS SEPTIC ARTHRITIS

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Abstract. There are reports of a correlation between high adenosine deaminase (ADA) levels in body fluid and tuberculosis (TB) infection, but none have evaluated synovial fluid ADA and TB arthritis. The objectives of this study were to determine the proper cut-off level for synovial fluid adenosine deaminase (SF-ADA) and the sensitivity and specificity of SF-ADA to diagnose TB arthritis. Between January 2006 and December 2007, SF-ADA were determined using the modified Giusti's method on patients over 15 years of age with clinically suspected TB arthritis or having an unknown etiology of their arthritis. Synovial fluid culture for TB was performed in all patients as a gold standard test. Forty cases were included in the study, with a female to male ratio of 1.7:1 and a mean age of 52.3±17.4 years (range, 16-80). The median duration of symptoms was 60 days. The prevalence of TB arthritis was 16.7% (6 cases) while the remaining cases were rheumatoid arthritis (8), non-TB bacterial septic arthritis (3), and miscellaneous (23). The mean SF-ADA levels in patients with TB arthritis and non-TB arthritis were 35.7±10.4 (range, 20-51) and 15.4±9 (range, 2-34) U/l, respectively. The cut-off value for the diagnosis of TB arthritis was 31 U/l, with a sensitivity of 83.3% (95%CI 35.9-99.6), a specificity of 96.7% (95%CI 82.8-99.9) and an agreement Kappa of 0.8 ($p<0.001$). SF-ADA levels higher than 31 U/l were highly correlated with a diagnosis of TB arthritis, with a high sensitivity and specificity. SF-ADA may be considered as a less invasive and time-consuming diagnostic tool for TB arthritis.

Keywords: tuberculosis, *Mycobacterium tuberculosis*, septic arthritis, tuberculous septic arthritis, adenosine deaminase, synovial fluid

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INTRODUCTION

Tuberculous septic arthritis (TB arthritis) is in the differential diagnosis of chronic arthritis, particularly chronic monoarthritis. The gold standard for diagnosing TB arthritis is a positive synovial

fluid acid-fast smear and/or a fluid culture positive for *Mycobacterium tuberculosis*. The yield for diagnosing TB arthritis using a positive fluid smear is low (Foocharoen *et al*, 2010) and a fluid culture for *M. tuberculosis* is time-consuming (6-8 weeks). An invasive test, such as a synovium biopsy, is helpful when making a diagnosis. Caseous granulomatous change, identified by synovium biopsy, is the classical finding in TB arthritis; however, most patients have to stay in the hospital after undergoing a synovium biopsy and some develop post-operative complications, such as hemarthrosis and septic arthritis.

Adenosine deaminase (ADA) is an enzyme that converts adenosine to inosine and deoxyadenosine to deoxyinosine via deamination (Aldrich *et al*, 2000). There are two ADA isoenzymes; ADA1 and ADA2. ADA1 can be found in all cell types, while ADA2 is found in monocytes and lymphocytes. A high ADA1 level indicates cell injury, while a high ADA2 level indicates monocytes and lymphocytes have been stimulated (Kurata, 1995; Pérez-Rodríguez *et al*, 2000). The ADA level, particularly ADA2, is useful for detecting diseases which stimulate lymphocyte and/or monocyte activity, such as tuberculosis. There are reports of a correlation between high ADA levels in body fluid (pleural, pericardial, peritoneal and cerebrospinal) and TB infection (Yuksel and Akolu, 1988; Reechaipichitkul *et al*, 2001; Riquelme *et al*, 2006; Tuon *et al*, 2006; Zaric *et al*, 2008; Krenke and Korczynski, 2010; Moghtaderi *et al*, 2010), but none about a correlation between ADA levels in synovial fluid and TB arthritis. ADA level in synovial fluid were evaluated by Zamani *et al* (2010) to differentiate inflammatory from non-inflammatory arthritis. The results show a high ADA level in synovial fluid could be a marker of septic

arthritis (not TB arthritis) rather than RA, crystal induced arthritis or osteoarthritis (Zamani *et al*, 2010). Additionally, a high level of ADA1 in the synovial fluid in RA patients correlated with synovial fluid matrix metalloproteinase enzyme which leads to cartilage and joint destruction (Iwaki-Ekawa *et al*, 2001; Nakamachi *et al*, 2003).

The hypothesis of our study was a high ADA level in the synovial fluid is correlated with TB arthritis. The objectives of our study were a) to evaluate a cut-off level of synovial fluid ADA to diagnose TB arthritis and b) to evaluate the sensitivity and specificity of this diagnosis.

MATERIALS AND METHODS

Between January 2006 and December 2009, the ADA level in synovial fluid was checked in patients ≥ 15 years of age, with clinically suspected TB arthritis or unknown etiologies of arthritis that were candidates for arthrocentesis. Synovial fluid and synovium culture for TB were performed in all of these patients.

Synovial fluid was kept in sterile heparin until measured for ADA using the modified Giusti's method (Giusti, 1974). Synovial fluid for *Mycobacterium tuberculosis* culture and routine bacteria culture were obtained by an orthopedic surgeon on admission and the laboratory testing was performed at the Microbiology Unit.

Diagnostic criteria

TB arthritis was defined as synovial fluid or synovium culture positive for *Mycobacterium tuberculosis*. Rheumatoid arthritis (RA) was defined by either the American College of Rheumatology criteria or synovium pathology compatible with RA. Septic arthritis was defined as synovial fluid culture positive for bacteria.

Crystal-induced arthritis was defined as the presence of monosodium urate or calcium pyrophosphate dehydrate in the synovial fluid or synovium. If the clinical characteristics and laboratory results did not fill the diagnostic criteria for a specific rheumatic disease, undifferentiated arthritis (UA) was diagnosed (Harrison *et al*, 1998).

The data were analyzed using STATA version 11.0. Continuous data were presented as a mean plus SD and categorical data were presented as a number and percentage. Nonparametric Kruskal-Wallis was used to compare means. The ROC curve was run and the sensitivity, specificity and Kappa for agreement were calculated.

Ethical considerations

The current study was approved by the Ethics Committee of Khon Kaen University. All patients gave written informed consent before any synovial fluid and/or tissue were sampled.

RESULTS

Forty patients who had clinically suspected TB arthritis or arthritis of unknown etiology were evaluated for synovial fluid ADA. Females outnumbered males (25 *vs* 15 cases). The average age at presentation was 51±17.6 years (range, 16-80) and the median duration of arthritis was 60 days (range, 7-360). Twenty-five cases had an underlying disease. Rheumatoid arthritis (RA) was the most common pre-existing disease (8 cases), followed by diabetes mellitus (6 cases) (Table 1). The majority of cases presented with monoarthritis, the knee joint being the most commonly affected joint (29 cases), followed by the ankle (12 cases) and the wrist (10 cases) (Table 1).

Table 1
Clinical presentation.

| Data | N = 40 (%) |
|---------------------------------|------------|
| Underlying disease | |
| Rheumatic disease | 13 (32.5) |
| Rheumatoid arthritis | 8 |
| Systemic lupus erythematosus | 2 |
| Systemic sclerosis | 1 |
| Polymyositis | 1 |
| Gout | 1 |
| Non rheumatic disease | 12 (30) |
| Diabetes mellitus | 6 |
| Chronic kidney disease | 3 |
| Cancer of nasopharynx | 2 |
| Thalassemia | 1 |
| Thyroid nodule | 1 |
| Number joint involvement | |
| Monoarthritis | 23 (57.5) |
| Oligoarthritis | 7 (17.5) |
| Polyarthritis | 10 (25) |
| Chest radiography | |
| Normal | 30 (75) |
| Upper lobe infiltration | 6 (15) |
| Interstitial infiltration | 4 (10) |
| Synovial fluid gross appearance | |
| Turbid | 28 (70) |
| Serosanguineous | 8 (20) |
| Pus | 4 (10) |

Evaluation of synovial fluid ADA was done in 36 cases, while fluid hyperviscosity prevented ADA evaluation in the remaining cases. Seven patients were diagnosed as having TB arthritis by synovial fluid or synovium culture for *Mycobacterium tuberculosis*; however, 1 of these cases was not evaluated for synovial fluid ADA because the fluid was purulent. The prevalence of TB arthritis in our study was 16.7%. Other final diagnoses were RA (8 cases), non-TB septic arthritis (3), crystal-induced arthritis (3) and miscellaneous (19). Of the non-TB septic arthritis cases, 1 was diagnosed as having *Escherichia coli*

Table 2
Detailed report of sensitivity and specificity.

| Cut-off ADA level | Sensitivity % | Specificity % | Classified % | LR+ | LR- |
|-------------------|------------------|------------------|-----------------|---------|--------|
| ≥ 2 | 100.0 | 0.0 | 16.7 | 1.0000 | |
| ≥ 3 | 100.0 | 6.7 | 22.2 | 1.0714 | 0.0000 |
| ≥ 4 | 100.0 | 13.3 | 27.8 | 1.1538 | 0.0000 |
| ≥ 5 | 100.0 | 16.7 | 30.6 | 1.2000 | 0.0000 |
| ≥ 6 | 100.0 | 20.0 | 33.3 | 1.2500 | 0.0000 |
| ≥ 7 | 100.0 | 23.3 | 36.1 | 1.3043 | 0.0000 |
| ≥ 8 | 100.0 | 26.7 | 38.9 | 1.3636 | 0.0000 |
| ≥ 9 | 100.0 | 30.0 | 41.7 | 1.4286 | 0.0000 |
| ≥ 12 | 100.0 | 33.3 | 44.4 | 1.5000 | 0.0000 |
| ≥ 13 | 100.0 | 36.7 | 47.2 | 1.5789 | 0.0000 |
| ≥ 14 | 100.0 | 40.0 | 50.0 | 1.6667 | 0.0000 |
| ≥ 15 | 100.0 | 43.3 | 52.8 | 1.7647 | 0.0000 |
| ≥ 16 | 100.0 | 46.7 | 55.6 | 1.8750 | 0.0000 |
| ≥ 17 | 100.0 | 50.0 | 58.3 | 2.0000 | 0.0000 |
| ≥ 18 | 100.0 | 60.0 | 66.7 | 2.5000 | 0.0000 |
| ≥ 20 | 100.0 | 63.3 | 69.4 | 2.7273 | 0.0000 |
| ≥ 23 | 83.3 | 73.3 | 75.0 | 3.1250 | 0.2273 |
| ≥ 24 | 83.3 | 76.7 | 77.8 | 3.5714 | 0.2174 |
| ≥ 25 | 83.3 | 80.0 | 80.6 | 4.1667 | 0.2083 |
| ≥ 26 | 83.3 | 83.3 | 83.3 | 5.0000 | 0.2000 |
| ≥ 28 | 83.3 | 90.0 | 88.9 | 8.3333 | 0.1852 |
| ≥ 31 | 83.3 | 96.7 | 94.4 | 25.0000 | 0.1724 |
| ≥ 33 | 66.7 | 96.7 | 91.7 | 20.0000 | 0.3448 |
| ≥ 34 | 50.0 | 96.7 | 88.9 | 15.0000 | 0.5172 |
| ≥ 38 | 50.0 | 100.0 | 91.7 | | 0.5000 |
| ≥ 41 | 33.3 | 100.0 | 88.9 | | 0.6667 |
| ≥ 51 | 16.7 | 100.0 | 86.1 | | 0.8333 |
| > 51 | 0.0 | 100.0 | 83.3 | | 1.0000 |

ADA, Adenosine deaminase

LR+, likelihood ratio for a positive test result

LR-, Likelihood ratio for a negative test result

septic arthritis, 1 with *Burkholderia pseudomallei* septic arthritis and 1 with *Salmonella* group D septic arthritis.

The mean synovial fluid ADA level in the TB arthritis, RA, non-TB septic arthritis, crystal-induced arthritis, and miscellaneous cases were: 35.7 ± 10.4 (20-51), 16.8 ± 11.7 (3-34), 23.7 ± 5.9 (17-28), 15 ± 12.1 (2-26), and 12.8 ± 7.1 (2-24), respectively (Fig 1). The

synovial fluid ADA levels in TB arthritis cases were significantly higher than in those with RA, non-TB septic arthritis, crystal-induced arthritis and miscellaneous ($p=0.007$).

After evaluating the ROC curve (Fig 2), the sensitivity and specificity for synovial fluid ADA for diagnosing TB arthritis were obtained (Table 2). A synovial fluid

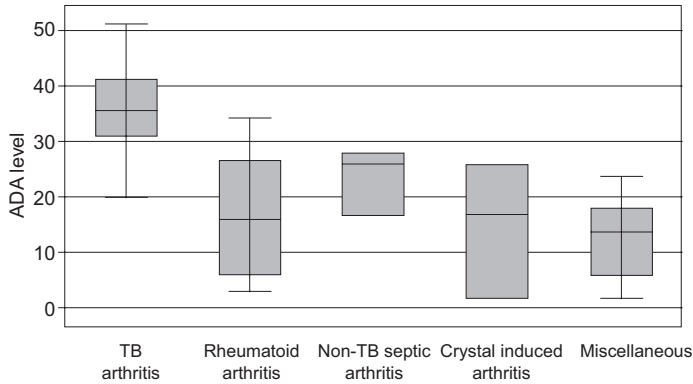


Fig 1–Comparison of synovial fluid ADA levels.

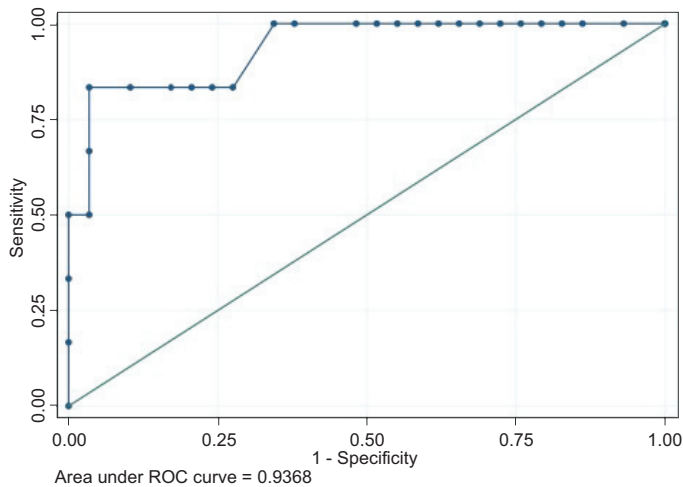


Fig 2–ROC curve.

ADA level of 31 U/l gave a sensitivity of 83.3% (95%CI 35.9-99.6) and a specificity of 96.7% (95%CI 82.8-99.9) with a Kappa of agreement of 0.8 ($p < 0.001$).

DISCUSSION

Both serum and synovial fluid ADA have been studied in patients with rheumatic disease. Most of the results show a high synovial fluid ADA level can help to differentiate inflammatory diseases (RA, crystal-induced arthritis and septic ar-

thritis) from non-inflammatory diseases (osteoarthritis) (Yuksel and Akolu, 1988; Erer *et al*, 2009; Zamani *et al*, 2010). In patients with RA, an elevated serum ADA level can indicate a TB infection, especially in RA patients who have received antitumor necrotic alpha (Erer *et al*, 2009).

Our study shows synovial ADA might be useful for diagnosing TB arthritis, since a high synovial fluid ADA level was associated with intra-articular TB infection. With reference to the gold standard test, the specificity and sensitivity of the ADA level were $> 95\%$ and $> 80\%$, respectively. Synovial ADA might be an alternative tool for the early diagnosis of TB arthritis since it is less time-consuming than TB culture and less invasive than a synovium biopsy.

Synovial fluid ADA levels in non-TB septic arthritis patients have been reportedly higher than in other types of inflammatory arthritis (Zamani *et al*, 2010) but not as high as in TB arthritis. *Mycobacterium tuberculosis* is an intracellular organism which can exist for many years in a latent phase in a macrophage. It has the potential to stimulate monocyte and lymphocyte function, a cellular immune response during reactivation (Schluger and Rom, 1998). Since the ADA level increases after monocyte and lymphocyte stimulation, one would expect to find high levels of ADA during reactivation. The cellular immune response does not predominate in non-TB septic arthritis as it does in TB arthritis, which may explain why synovial fluid ADA levels in TB arthritis are higher

than in non-TB septic arthritis.

Even though synovial fluid ADA level may be useful for diagnosing TB arthritis, the evaluation was limited by synovial hyperviscosity. In four of our cases, the synovial fluid ADA level could not be evaluated because of pus formation and in one, the synovial fluid culture was positive for *Mycobacterium tuberculosis*. Culturing synovial fluid and/or synovium for *Mycobacterium tuberculosis* is still the best tool for diagnosing TB arthritis when there is clinically-suspected TB arthritis and there is pus formation in the fluid.

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