

Diagnosis of Spinal Infection with Alpha-defensin Lateral Flow Test: A Preliminary Report

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Abstract:

Introduction: The alpha-defensin lateral flow test has been used in periprosthetic joint infection as a diagnostic support tool because of its simplicity and speed. However, the test has not been used to diagnose spinal infections. The purpose of this study was to investigate the efficacy of the alpha-defensin lateral flow test for diagnosing spinal infections.

Methods: The subjects were 11 patients who were suspected of having spinal infections from October 2019 to August 2021 and underwent biopsies at a single institution. There were nine male and two female patients, with an average age of 60.7 (14-87) years. For diagnosing infection, the patient's consent for biopsy was obtained, and the sample was collected by computed tomography-guided aspiration biopsy or open biopsy at the site considered to be a possible abscess. The samples were subjected to a bacterial culture test, an acid-fast bacillus culture test, and an alpha-defensin lateral flow test (Synovasure[®] lateral flow test; Zimmer Biomet, IN, USA).

Results: Of the 11 suspected spinal infections, the alpha-defensin lateral flow test was positive in 8 cases, negative in 2 cases, and undeterminable in 1 case. Of the 10 cases excluding the undeterminable case, the definitive diagnosis was 9 cases of spinal infection (spondylitis: 6 cases, spinal implant infection: 3 cases) and 1 case of vertebral body fracture. The alpha-defensin lateral flow test demonstrated a sensitivity of 88.9%, a specificity of 100%, a positive predictive value of 100%, and a negative predictive value of 50%. The biopsy sample culture test demonstrated a sensitivity of 77.8%, a specificity of 100%, a positive predictive value of 100%, and a negative predictive value of 33.3%.

Conclusions: We suggested that the alpha-defensin lateral flow test might be useful as a diagnostic support tool for spinal infections.

Keywords:

alpha-defensin, spinal infection, spondylitis, spinal implant infection, alpha-defensin lateral flow test

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Introduction

Alpha-defensins are antibacterial peptides that are released from neutrophils in response to pathogens. They act to depolarize the cell membrane of the pathogen¹⁾. Therefore, the presence of alpha-defensins in human joints and tissues can be used as a sensitive test for diagnosing infection. Two methods for measuring alpha-defensins are commercially available: one is the enzyme-linked immunosorbent assay-based alpha-defensin immunoassay test (Synovasure[®] alpha-defensin immunoassay; Zimmer Biomet, IN, USA) and the other is the alpha-defensin lateral flow test (Synovasure[®] lateral flow test; Zimmer Biomet, IN, USA)²⁾. The alpha-

defensin immunoassay test has a sensitivity of 97%-100% and a specificity of 95%-98% in periprosthetic joint infection (PJI)³⁻⁶⁾, but it has the drawback of requiring 24 h to perform. On the other hand, the alpha-defensin lateral flow test can yield a qualitative result within 10 min and was developed for the purpose of diagnosing PJI.

According to the guideline of the Infectious Diseases Society of America (IDSA), tests for erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), blood culture, and spinal magnetic resonance imaging (MRI) are recommended for patients with suspected spinal infection⁷⁾. It is also recommended to perform culture tests for brucellosis bacteria, fungi, and acid-fast bacilli. Although PJI has a definition^{8,9)},

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it is ultimately advisable to seek comprehensive judgment by infectious disease specialists or spine surgeons in cases of spinal infection⁷⁾.

When spinal infections such as spondylitis, discitis, vertebral osteomyelitis, epidural abscess, surgical site infection, or postoperative spinal implant infection are suspected, it is not uncommon for bacteria to be undetectable. In addition, the time required for culture test results can make diagnosis difficult or delay treatment. The alpha-defensin lateral flow test has been used in PJI as a diagnostic support tool because of its simplicity and speed. However, to date, the alpha-defensin lateral flow test has not been used to diagnose spinal infections. The purpose of this study was to investigate the efficacy of the alpha-defensin lateral flow test for diagnosing spinal infections.

Materials and Methods

The institutional review board approved this study. The subjects were 11 patients who were suspected of having spinal infections from October 2019 to August 2021 and underwent biopsies and alpha-defensin lateral flow tests at a university hospital. Since the supply of test kits was restricted by the coronavirus disease (COVID-19) pandemic, the other seven patients could not be tested with the alpha-defensin lateral flow test during the study period. There were nine male and two female patients, with an average age of 60.7 (14-87) years. For diagnosing infection, the patient's consent for biopsy was obtained, and the sample was collected by computed tomography (CT)-guided aspiration biopsy or open biopsy at the site considered to be a possible abscess. Three patients were treated with antibiotics at the time of biopsy. The samples were subjected to a bacterial culture test, an acid-fast bacillus culture test, and an alpha-defensin lateral flow test (Synovasure[®] lateral flow test; Zimmer Biomet, IN, USA). When a blood clot or a highly viscous sample was collected, it was diluted with a small amount of saline and tested. According to the IDSA guideline, the diagnosis of spinal infection was completed by observing the changes over 2 months or more in the bacterial/acid-fast bacillus culture test, X-ray image, MRI, CT, white blood cell count (WBC), and CRP⁷⁾.

Results

Of the 11 suspected spinal infections, the alpha-defensin lateral flow test was positive in 8 cases, negative in 2 cases, and undeterminable in 1 case. In this undeterminable case, the test kit's line did not appear as a line, rendering the judgment invalid. Of the 10 cases excluding the undeterminable case, the definitive diagnosis was 9 cases of spinal infection (spondylitis: 6 cases, spinal implant infection: 3 cases) and 1 case of vertebral body fracture (Table 1). Two cases from the cohort are presented in detail below.

The alpha-defensin lateral flow test demonstrated a sensitivity of 88.9%, a specificity of 100%, a positive predictive

value of 100%, and a negative predictive value of 50%. The biopsy sample culture test demonstrated a sensitivity of 77.8%, a specificity of 100%, a positive predictive value of 100%, and a negative predictive value of 33.3%.

Case presentation

Case 1: An 87-year-old man with a history of diabetes, arteriosclerosis obliterans, and sick sinus syndrome presented with a 1-month history of low back pain. His blood sample revealed a WBC of 7200/ μ L, CRP of 4.20 mg/dL, and ESR of 53 mm/h. CT images showed a vertebral endplate distraction at L2/3 and paraspinal abscess. MRI was not available because the patient had a cardiac pacemaker. The fluid sample was collected by CT-guided aspiration biopsy of the psoas major muscle and intervertebral disc. Both bacterial and acid-fast bacillus cultures of the sample were negative, but the alpha-defensin lateral flow test was positive (Fig. 1). Based on these results, we made a diagnosis of pyogenic spondylitis.

Case 7: An 81-year-old man presented with back pain after a fall. His blood sample test revealed a WBC of 8400/ μ L, CRP of 4.82 mg/dL, and ESR of 23 mm/h. The enhanced CT images showed a marginal contrast effect around the T10 vertebral body and fluid pooled with air. A short tau inversion recovery MRI showed increased signal intensity at the T9/10 intervertebral disc. CT-guided aspiration biopsy of the T9/10 intervertebral disc was performed. The biopsy sample used for analysis consisted of fluid that had been washed with saline and mixed with blood. Both bacterial and acid-fast bacillus cultures of the sample were negative. The alpha-defensin lateral flow test was also negative (Fig. 2). Based on these results, we made a diagnosis of thoracic vertebral fracture with diffuse idiopathic skeletal hypertrophy.

Discussion

The alpha-defensin lateral flow test for PJI has been reported to be an excellent test with a sensitivity of 67%-77% and a specificity of 82%-94%¹⁰⁻¹²⁾. The current study showed a sensitivity of 88.9% and a specificity of 100% for spinal infections. The lateral flow test was able to support diagnosis of spinal infection even when the bacterial culture test was negative. To the best of our knowledge, this is the first report to show that the alpha-defensin lateral flow test is useful for diagnosing spinal infections.

Our results revealed several factors that can influence the results of the alpha-defensin lateral flow test. Samples mixed with saline or contrast may yield false negative results. In addition, hemoglobin, triglycerides, and bilirubin (conjugated type and non-conjugated type) act as interfering substances, and false negatives or judgment invalidity can result from blood contamination of the sample. It had been speculated that the usefulness of the alpha-defensin lateral flow test in spinal infections was inferior to that in PJI because of these inhibitors, but the usefulness was equivalent in this

Table 1. Biopsy Sample Culture, Alpha-defensin Lateral Flow Test, and Definitive Diagnosis of Suspected Spinal Infections.

Case	Age	Sex	Site of suspected infection	Comorbidity	Period of illness	Antibiotics at the time of biopsy	Blood culture	Biopsy method	Biopsy sample culture	Alpha-defensin lateral flow test	Definitive diagnosis
1	87	M	L2/3 disc, vertebrae	DM, ASO, SSS	3W	None	(-)	CT-guided	(-)	Positive	SI (SL)
2	76	F	L4/5 disc, vertebrae	TAA, PE	4W	CEZ	<i>Staphylococcus aureus</i>	CT-guided	<i>Staphylococcus aureus</i>	Positive	SI (SL)
3	71	M	T6/7 disc, vertebrae	DM	4W	CEZ	(-)	CT-guided	<i>Staphylococcus aureus</i>	Positive	SI (SL)
4	35	M	C6/7 disc, vertebrae, L5/S1 disc, vertebrae	AD	7W	None	<i>Staphylococcus aureus</i> , <i>Staphylococcus epidermidis</i>	CT-guided	<i>Staphylococcus aureus</i>	(-)	SI (SL)
5	12	F	T12/L1 disc, vertebrae	AD	4W	None	(-)	CT-guided	<i>Staphylococcus aureus</i>	Positive	SI (SL)
6	68	M	L4/5 disc, vertebrae	DM, myelofibrosis	24W	None	(-)	CT-guided	<i>Mycobacterium abscessus</i>	Positive	SI (SL)
7	81	M	T9/10 disc, vertebrae	SCI	1W	None	(-)	CT-guided	(-)	(-)	Fracture
8	66	M	T2–L4 posterior implant	Schizophrenia	6W	None	(-)	Open	(-)	Positive	SI (SII)
9	32	M	L1–L5 posterior implant	AD	2W	None	(-)	Open	<i>Escherichia coli</i> , <i>Bacteroides fragilis</i>	Positive	SI (SII)
10	64	M	T2–S2 posterior implant	PD	2W	CEZ	(-)	Open	<i>Staphylococcus capitis</i>	Positive	SI (SII)

DM, diabetes mellitus; ASO, arteriosclerosis obliterans; SSS, sick sinus syndrome; TAA, thoracic aortic aneurysm; PE, pulmonary emphysema; AD, atopic dermatitis; SCI, spinal cord injury; (-), negative; W, week (s); CEZ, Cefazolin; CT, computed tomography; SI, spinal infection; SL, spondylitis; SII, spinal implant infection

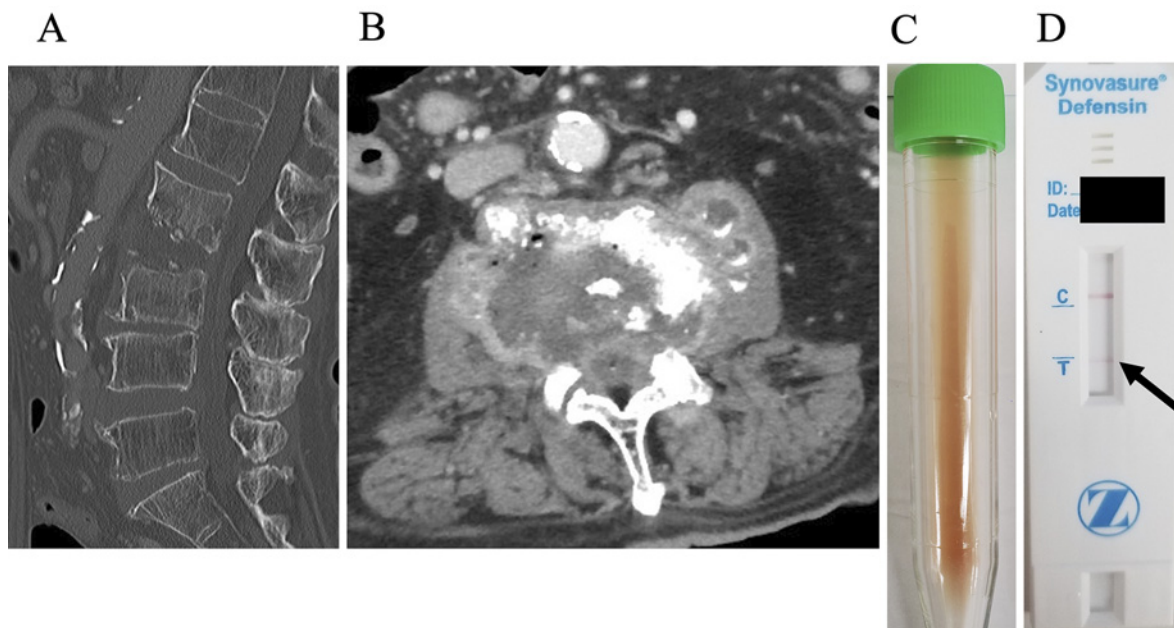


Figure 1. This case is an 87-year-old man (Case 1). (A) Sagittal CT image showed vertebral endplate distraction at L2/3. (B) Axial image of enhanced CT showed paraspinal lesion at L2/3. (C) The fluid sample was collected by CT-guided aspiration biopsy of the psoas major muscle and intervertebral disc. (D) The alpha-defensin lateral flow test of the sample was positive (arrow).

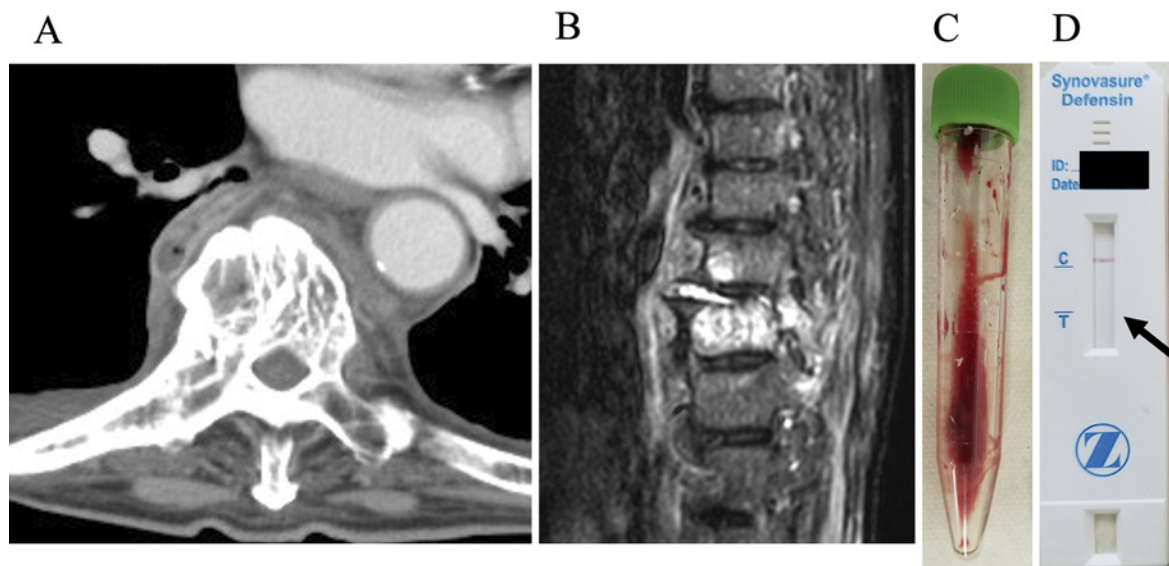


Figure 2. This case is an 81-year-old man (Case 7). (A) The enhanced CT images showed marginal contrast effect lesion around the T10 vertebral body and fluid pooled with air. (B) A short tau inversion recovery MRI showed increased signal intensity at the T9/10 intervertebral disc. (C) CT-guided aspiration biopsy of the T9/10 intervertebral disc was performed. The biopsy sample used for analysis consisted of fluid that had been washed with saline and mixed with blood. (D) The alpha-defensin lateral flow test was negative (arrow).

study.

Interestingly, the alpha-defensin lateral flow test was positive for acid-fast bacillus infection. Case 6 in this study was a spinal infection caused by the relatively uncommon *Mycobacterium abscessus*, and the alpha-defensin lateral flow test was positive. Because acid-fast bacillus culture results take longer than for common bacteria, the addition of the alpha-defensin lateral flow test may have been useful in estimating the presence of acid-fast bacillus infection.

For patients on antibiotic treatment, the alpha-defensin lateral flow test may be useful as a diagnostic tool. A multi-institutional study found that starting antibiotic treatment for PJI before obtaining a diagnosis did not appear to be linked to lower median alpha-defensin levels. The study also showed that alpha-defensin levels in synovial fluid might be maintained even when patients underwent antibiotic treatment before undergoing diagnostic testing¹³. In the present study, three patients were treated with antibiotics at the time of biopsy, and the alpha-defensin lateral flow test could detect infection even in the presence of antibiotics. We assume that the alpha-defensin lateral flow test is effective even in the presence of antibiotics, based on our study and previous studies.

The advantages of the alpha-defensin lateral flow test in diagnosing spinal infections were that the test was completed in 10 min, and that infection was detectable even in the presence of antibiotics¹³. This test was especially useful for confirming spinal implant infections and for vertebral body and intervertebral disc injuries that are suspected of being infected. On the other hand, the disadvantages were that the test could not be performed when it was difficult to collect a sample, that the type of bacteria could not be iden-

tified, and that the cost of the test kit is expensive (50,000 Japanese yen, approximately US\$ 450).

There were several limitations in this study. Since the number of cases was as small as 11 and the number of negative cases was particularly small, we thought that the negative predictive value might be higher. To confirm these results, it will be necessary to increase the number of cases and examine in detail. The research was not performed consecutively. The supply of test kits was restricted by the COVID-19 pandemic within the period covered. We tried it when the test kit was ready. Both postoperative spinal implant infection and native vertebral osteomyelitis were included in this study. Because the two conditions are mixed, the number of cases needs to be increased, and each condition must be examined independently. Since the alpha-defensin lateral flow test is expensive, verifying cost-effectiveness will be a future issue. Since the diagnosis of spinal infections was comprehensive, the alpha-defensin lateral flow test was employed as a diagnostic support tool.

The alpha-defensin lateral flow test was used to obtain rapid proof of infection in the spinal region when pyogenic spondylitis or spinal implant infection was suspected. We suggested that the alpha-defensin lateral flow test might be useful as a diagnostic support tool for spinal infections. Since this paper is a preliminary report, it is necessary to increase the number of cases and verify the usefulness.

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Informed Consent: The opt-out method was adopted to obtain informed consent for publication from the subjects.

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