Specific pathomorphological appearance of tuberculous spondylitis and the results of immunohistochemical expression levels of CD 45 and Ki 67 markers

Makhmudova Z.P., Nazirov P.Kh., Sanoev B.A.,
Republican Specialized Scientific and Practical Medical Center of Phthisiatry and Pulmonology named after Sh. A. Alimov, Bukhara State Medical Institute

Abstract. This scientific article presents the data obtained based on the pathomorphological examination of operative-histological materials from patients with tuberculous spondylitis, based on the pathomorphological characteristics of tuberculous spondylitis and the level of expression of immunohistochemical markers CD 45 and Ki 67.

Keywords: Tuberculosis, spondylitis, marker, immunohistochemistry, spine, biopsy.

The purpose of the study: Determination of pathomorphological changes of bone tissue and immunohistochemical expression of CD 45 and Ki 67 markers in patients with tuberculous spondylitis.

Materials and methods: The study involves the study of histological micropreparations from bone tissue of 18 patients with tuberculous spondylitis of different age and sex using the hemotoxylin-eosin and Van-Gizon method with general histological and histochemical properties characteristic of the disease and determination of the expression level of immunohistochemical markers CD 45 and Ki 67.

Paraffin bricks made from tissue taken from 18 patients with tuberculous spondylitis were selected for immunohistochemical study. After the tissue fragments obtained for immunohistochemical staining were cut using a microtome with a thickness of 2-4 μm, the item was placed on glass and covered with a poly-L-lysine sealer from above. The obtained tissues were subjected to dehydration and dewaxing of the sections by avidin-biotin-immunoperoxidase method, dehydration after dewaxing, demasking after Ventana Benchmark XT, and staining with antibodies in an automated Roche Special System, Switzerland. The study showed that CD 45 and Ki 67, samples stained using antibodies, are program-expressed cells with a very high microburst frequency (QuPath-0.4.0, NanoZoomer Digital Pathology Image).

The expression level of Ki 67 (proliferative index), CD 45 was evaluated as a percentage. Marker expression staining was quantified by relative percentages and graded as mild, moderate, and strong expression as follows: 0 (no staining), 1+ (<20% of cells, weakly stained), 2+ (20–60% cells, moderately stained), was scored as 3+ (>60% cells, strongly stained).

Main part: Vertebral tuberculosis or tuberculous spondylitis is a specific infectious lesion of the spine, caused by mycobacteria. A granuloma, which is characteristic of this disease, is formed and damages the bone tissue, which leads to damage to the spine.

Pathomorphologic examination and analysis of micropreparations from operative and biopsy materials revealed the presence of tuberculosis-specific granulomas in most of the bone tissue, necrotic tissue in the center of the granuloma and around it lymphohistiocytic cells, epithelioid cells and between them large Pirogov-Langhans cells, whose nuclei are located in many peripheral areas, including in a diffuse state. Leukocytic infiltrates were also encountered (See Figure 1). It was found that bone tissue, bone lamina, is destroyed in the center of the inflammation focus, dissolved and destroyed. It appeared that the boundaries of the bone plates preserved to the periphery were indistinct, dissected into small small fragments, the bone lamina was thinned, the lamina was eroded from the inside, and the lacunae in the bone lamina were compacted, getting closer to each other.

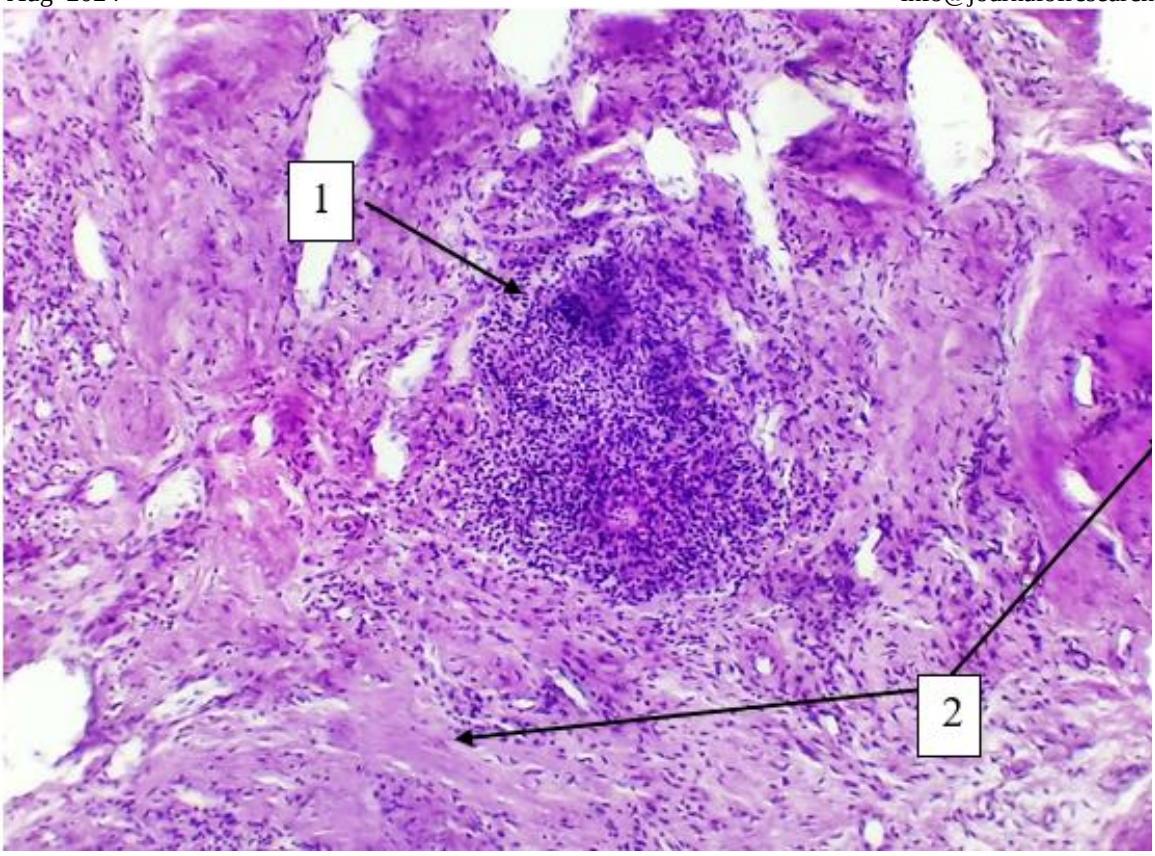


Figure 1. Tuberculous spondylitis. Hemotoxic eosin stained magnified 200 times. Specific granuloma characteristic of tuberculosis is identified in the given micropreparation (1). In the center of the granuloma, caseous necrosis and epithelioid cells and large Piragov Langhans cells with numerous nuclei are detected in the peripheral part. In the peripheral part, the bone plate is partially preserved, it is thinned and resorption processes are increased in the part of the preserved bone plate (2). The walls of blood vessels become thinner and signs of fullness appear. Sclerc, degenerative changes are detected around the granuloma.

In some tissues, osteocyte cells are not detected. In some parts, we could see the accumulation of osteoclast cells in the wall of partially preserved bone plates and increased resorption processes, in some parts of the bone tissue, hyperplasia of osteoblast cells in the peripheral part of preserved bone plates can be seen. In the structure of the tissue, some of the osteon channels are not detected, signs of fullness in the blood vessels in the tissue and local hemosiderosis foci are visible in some parts. An increase in the amount of mesenchymal cells and signs of infiltrative tumor among them were found. It was found that the amount of collagen fibers increased in the destroyed bone tissue, and the amount of collagen fibers around the blood vessel wall increased (see Fig. 2). Degenerative changes were manifested in the destroyed bone tissue, cell components and fibrous structures were proliferated and arranged in a disorderly manner, and at the border of healthy tissue, collagens with briquetting tissue components accumulated and were separated by capsule and sclerotic changes.

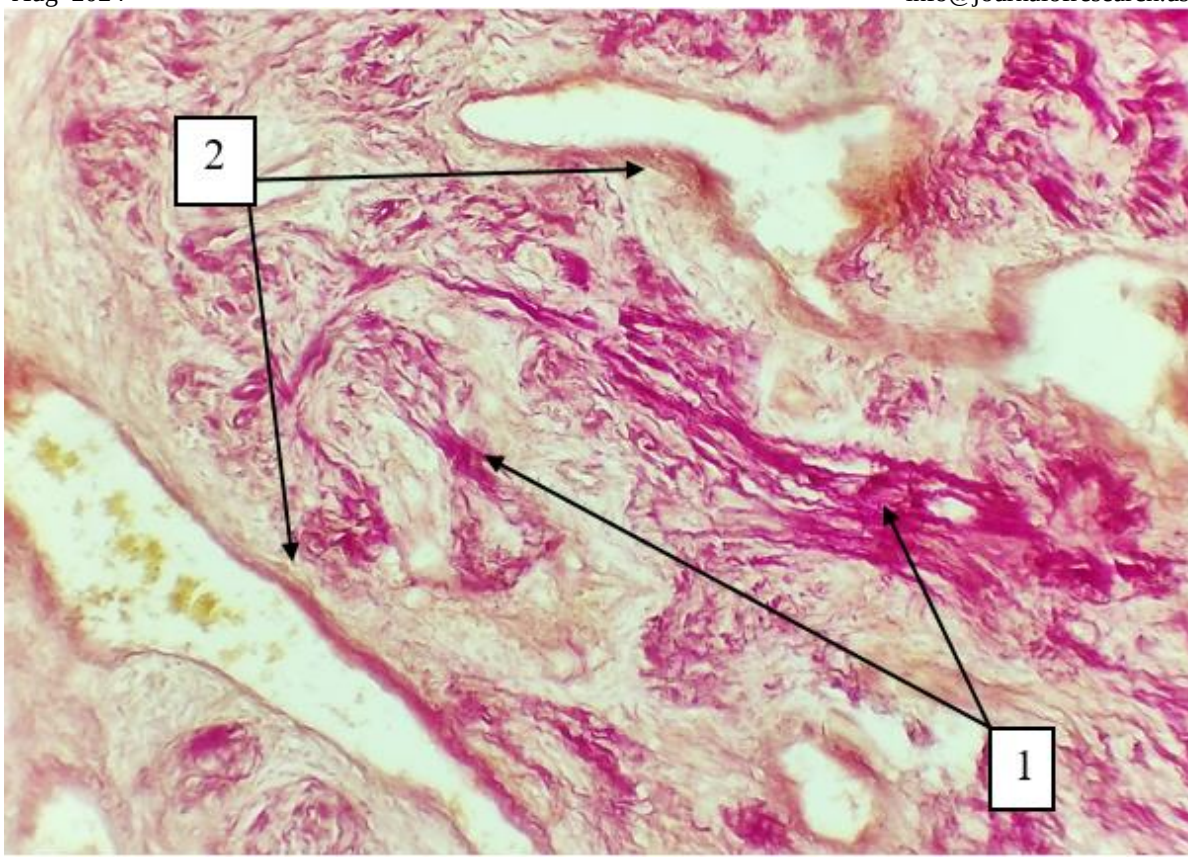
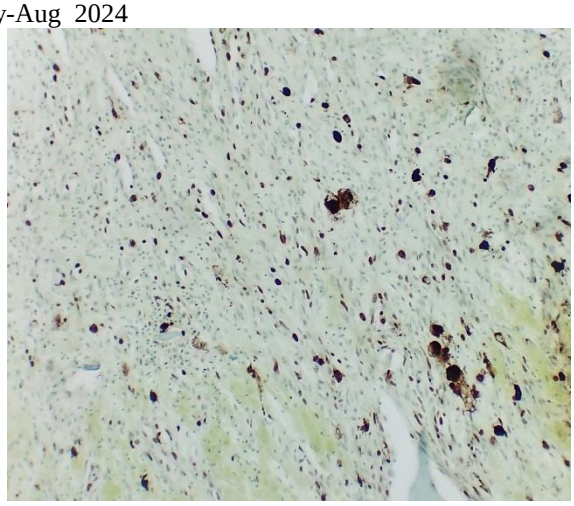
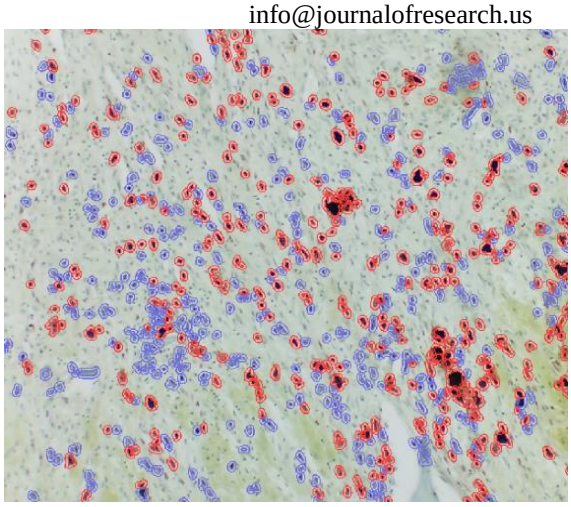


Figure 2. Tuberculous spondylitis. Magnified 200 times, stained by the Van Gizon method. Bone plates are eroded, thinned and separated into separate parts, their lacunae become denser and remain. In the destroyed bone tissue, fibrous structures are rough, coarse, disorderly. The amount of thin and fine collagen has increased in the walls of blood vessels.

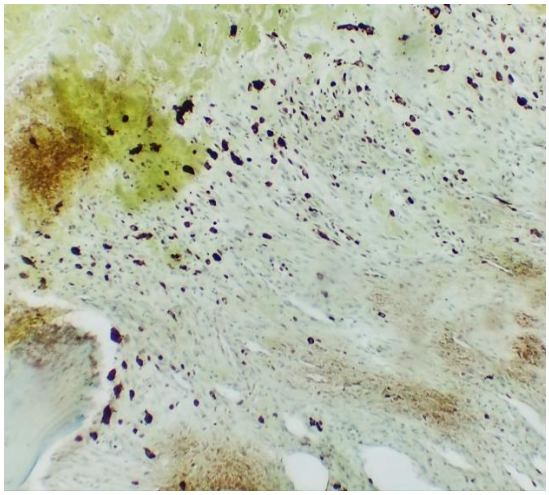
The level of expression of immunohistochemical Ki 67 markers of tuberculous spondylitis was studied. Ki 67 marker is used to evaluate the proliferative activity of cells, and it is also used to evaluate proliferative activity in tumor processes. This marker is evaluated in percentages and shows the degree to which the activity of dividing cells increases. A special protein expressing Ki 67 is located in the nucleus of the cell and is one of the materials important for cell proliferation.

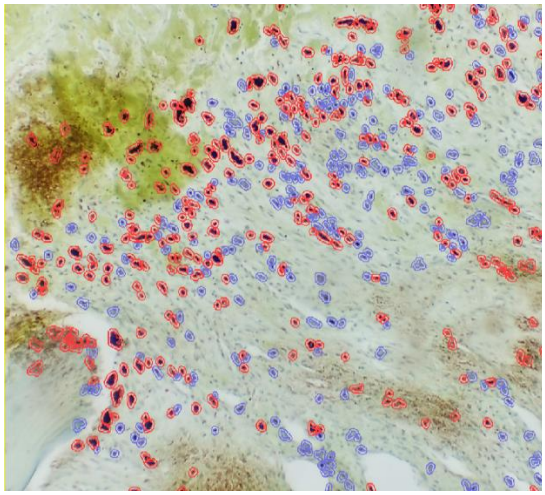




Total number of cells detected	898
Positive cells	474
Negative Cells	424
Positive Expression	52,7 %
General area	1046397 px^2

Figure 3: Expression of Ki 67 marker in bone tissue of patients with tuberculous spondylitis. Stained by DAB chromogenic method. The image is magnified 200 times. (QuPath-0.4.0, software evaluation. Cells with positive expression are in red) Positive expression 52,7%





Total number of cells detected	637
Positive cells	371
Negative Cells	266
Positive Expression	58,24 %
General area	1035300 px^2

Figure 4: Expression of Ki 67 marker in bone tissue of patients with tuberculous spondylitis. Stained by DAB chromogenic method. The image is magnified 200 times. (QuPath-0.4.0, software evaluation. Cells with positive expression are in red) Positive expression 58,24%

The expression levels of Ki 67 marker were studied by immunohistochemical analysis of bone tissue samples from patients with tuberculous spondylitis, and positive expression was found to be from 47% to 57%, that is, positive expression 2+. So, the average proliferative index was 52%

(2+). Proliferation was mainly due to mesenchymal cells around the inflammation focus, osteoclast cells and, to a lesser extent, osteoblast cells (see Figs. 3, 4).

The degree of expression of CD 45 immunohistochemical markers in tuberculous spondylitis was studied.

CD 45 marker is a leukocyte antigen of all types, this marker is a marker located on the surface membrane of all leukocytes. In hematopoietic cells, cells of the immune system, namely V-cells, T-cells and natural killer (NK) cells, monocytes and granulocytes are located on the surface. The CD 45 marker plays one of the important roles in the activation of immune cells and their differentiation into adulthood. This marker is used to determine which hematopoietic cells are T-cells and which are V-cells in pathological cases. At the same time, the expression of this marker increases in hematopoietic cells from immature cells to mature ones. It is highest in mature lymphocytes. There are several isoforms of this marker that are located on the membranes of T-cells, V-cells and monocytes. An example is the CD 45 RO epitopes for memory T lymphocytes.

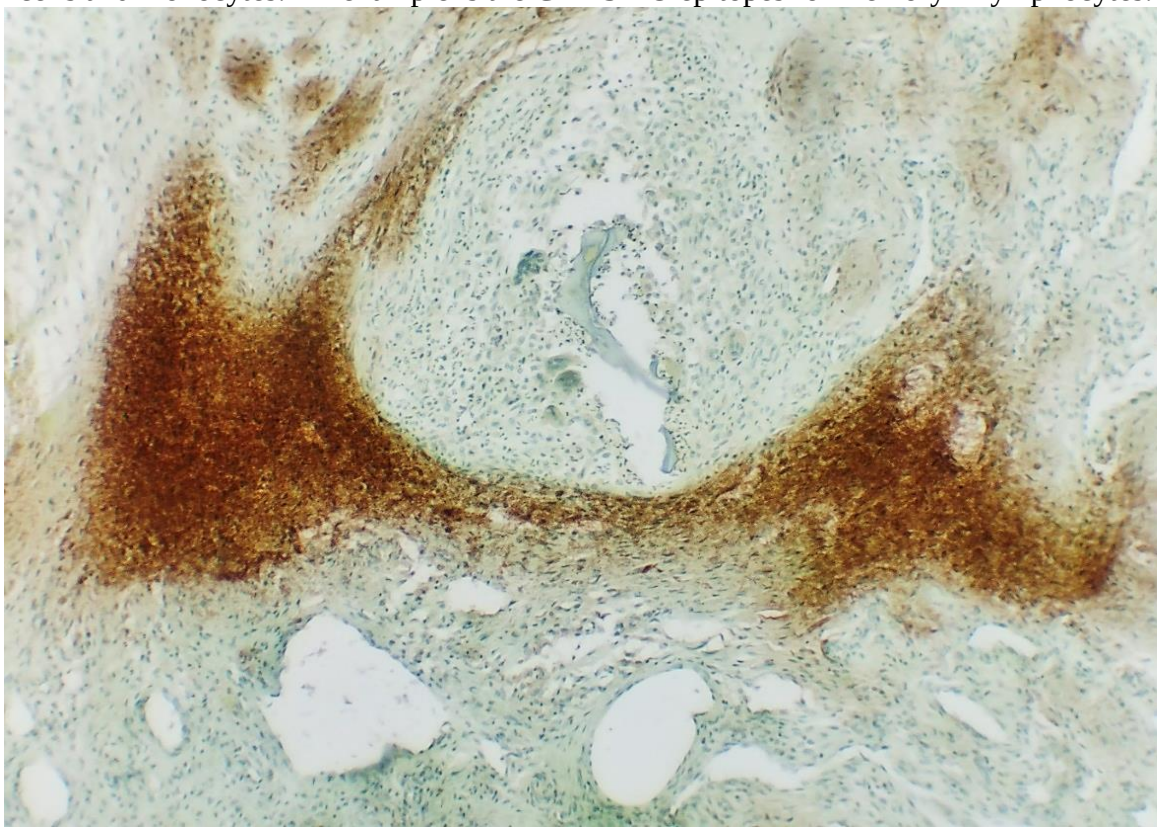


Figure 5. Expression of CD 45 marker in bone tissue infected with tuberculous spondylitis.

Stained by DAB chromogenic method. The image is magnified 200 times. Positive expression 23%

In immunohistochemical examination of preparations from bone tissue of patients with tuberculous spondylitis, the expression levels of CD 45 marker were determined as positive expression of 16% in 2 preparations, i.e., 1+, and from 22% to 31.8% in the rest, i.e., positive expression of 2+. Consequently, the leukocytic infiltrate averaged 27% (2+). It was found that leukocytic infiltrate mainly manifested as foci around the granuloma.

Conclusion: Thus, the following conclusions have been drawn in our study.

1. Macroscopic examination often reveals more damage to the pore substance of the vertebral body and almost less damage to the vertebral arch and vertebral outgrowths. The process was found to extend along the spinal surface (anteriorly, laterally, posteriorly) or vertebrae through the intervertebral disc. This suggests that intervertebral abscesses may form in patients and that the

process may spread upward or downward, spread along the lateral surface may cause paravertebral abscesses, spread along the posterior surface may compress the spinal cord causing neurologic symptoms and paralysis. Cold abscesses or fistulas have been observed in the lesion focus.

2. From the pathomorphological point of view, the appearance of specific granulomas characteristic of tuberculosis, that is, caseous necrosis in the center of the granuloma, epithelioid cells and large multinucleated Pirogov-Langhans cells were detected around it. It was found that the bone plates in the center of inflammation were broken, melted and destroyed, the bone plates preserved in the peripheral parts were divided into small parts and thinned, and the process of resorption increased in these plates. It was found that the walls of preserved blood vessels were thinned, hyperemia characteristic of inflammation, and signs of focal hemosiderosis were observed in some places.

3. The histochemical study confirmed that the processes of degenerative proliferation of cells and fibrous structures in destructive bone tissue, irregularly arranged, dense, thickened collagen fibers appear more often on the border of the inflamed focus and healthy tissue.

4. Based on the level of expression of the CD 45 marker in tuberculous spondylitis, it should be noted that the involvement of immune cells was on average 27% (2+). The expression level of Ki 67 marker in bone tissue was on average 52% (2+). This proliferative activity can be explained in this way, that is, it was found that it occurred due to the participation of more mesenchymal and osteoclast cells. This process proves that bone resorption and the **formation of fibrous structures are increased.**

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