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RESEARCH ARTICLE

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Analysis of laboratory and serological test results in patients with acute brucellosis during follow-up

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Abstract

Background: The laboratory test results and serum-specific antibodies of patients with acute brucellosis initial infection were followed up and analyzed.

Methods: 70 patients in Hohhot City, Inner Mongolia Autonomous Region, with acute brucellosis were followed up for 360 days. Serum samples were collected at 0, 15, 30, 60, 90, 180, and 360 days after diagnosis and analyzed by Rose Bengal plate test (RBPT), colloidal gold test paper (GICA), and test tube agglutination test (SAT). The serum-specific antibodies IgG and IgM were detected.

Results: RBPT results: False negative (-) gradually increased with the extension of the course of disease, with the largest change in 30–60 days after diagnosis, and the constituent ratio increased by 12.9%. GICA results: The false negative increased with the course of disease, and the constituent ratio of false negative was 20.0% after 180 days of diagnosis. SAT results: 1:100 positive showed a ladder like decrease with the increase in the course of disease, and the largest decrease was 90–180 days, with a decrease of 34.3% in the constituent ratio. 360 days after diagnosis, the constituent ratio of positive was only 14.3%. During the follow-up period, the IgG average value fluctuated and the average IgM value decreased.

Conclusion: The false-negative results of RBPT, GICA, and SAT increased with the course of disease, and the false-negative rates were higher than 20% after half a year. IgM level is beneficial to the early diagnosis of brucellosis, while IgG level is helpful to the judgment of brucellosis stage.

KEYWORDS
antibody, brucellosis, RBPT, SAT, serological

1 | INTRODUCTION

Brucellosis is a zoonotic disease caused by gram-negative bacilli, which is prevalent in the world, especially in developing countries.[1] Before the founding of the People's Republic of China, there was an epidemic of brucellosis, and it reappeared in the early 1990s in a sporadic state. Since 2020, the incidence of brucellosis in China has continued to be high.[2] Sheep is the main source of brucellosis in China, and lamb delivery by herdsmen or veterinarians is the main route of transmission. Fur, meat processing, milking, and so on can be infected through skin and mucous membrane. Eating sick animal meat, milk, and dairy products can be infected through digestive

tract. There is no persistent immunity, and reinfection is not uncommon. The mortality of patients with this disease was high, and because of the atypical symptoms, it is easy to turn into a chronic case, which causes serious complications and affects the health and quality of life of patients.[3] The damage caused by brucellosis is twofold. It can also affect the health of cattle and sheep, cause miscarriage and death, and seriously hinder the development of animal husbandry.[4] In recent years, with the continuous expansion of cattle and sheep breeding in the Inner Mongolia Autonomous Region, brucellosis continues to occur at a high rate, and the disease has become a serious public health problem in China.[5] The occurrence, development, and prognosis of brucellosis are complex, with various clinical manifestations and complicated diagnostic criteria. The diagnosis of brucellosis should be based on epidemiological history, clinical manifestation, and laboratory examination. Serological examination of brucellosis is simple and rapid, and can be used for large-scale screening, but its effect needs to be further evaluated.

In this study, patients with primary brucellosis infection were followed up, and their laboratory test results and serum-specific antibody titers were tracked and analyzed within 360 days after the diagnosis of brucellosis. By analyzing the changes in the above indicators, it provides a scientific basis for the screening and prevention of brucellosis.

2 | MATERIALS AND METHODS

2.1 | Source of information

From January 2019 to December 2019, 95 patients with acute brucellosis after initial infection diagnosed in Helin County, Damao Banner, and Wuchuan County of Baotou City, Inner Mongolia Autonomous Region. These patients were diagnosed as brucellosis in our Hospital. Inclusion criteria: (1) Patients diagnosed as brucellosis according to the diagnostic criteria of brucellosis.[6] (2)Acute attack of primary infection. (3) No antibiotics such as rifampicin were taken before diagnosis. Exclusion criteria: (1) Patients with tumors. (2)Patients with underlying diseases. (3) Patients who refused informed consent. (4) Patients who lost follow-up.

All patients took symptomatic treatment when symptoms appeared and were followed up for 360 days. 25 patients were lost to follow-up due to various reasons, and 70 patients were effectively tracked, with an effective rate of 73.7% (see Table 1 for the follow-up status of the three counties). During the follow-up, the basic information of the patient (name, age, gender, contact information, and detailed address of current residence) was recorded in detail, and serum samples were taken on the day of diagnosis (0 day), 15d, 30d, 60d, 90d, 180d, and 360d, and 490 serum samples were obtained. This study was approved by the Medical Ethics Committee of our Hospital.

2.2 | Methods

The diagnosis was made according to the latest WS 269–2019 "Diagnostic Criteria for Brucellosis".[6] (1) History of Epidemiology: Before the onset of the disease, the patient had a history of close contact with livestock or animal products, Brucella culture, or lived in an epidemic area, or had a close relationship with vaccine production, use, and research. (2) Clinical symptoms: Fever (including low fever), hyperhidrosis, fatigue, muscle, joint pain, and so forth. occurred for several days or even weeks. Most patients had enlarged lymph nodes, liver, spleen, and testis, and a few patients had various kinds of congestive rashes and jaundice; Most of the patients in the chronic stage showed bone and joint system damage. (3) Laboratory screening:Rose Bengal plate test (RBPT) and colloidal gold test paper (GICA) were used for preliminary screening. GICA test paper quality control line (C) and test line (T) had clearly visible red bands (+). (4) Serological test: The standard test tube agglutination test (SAT) was used for serum antibody testing to confirm the diagnosis, and if the titer was 1:100 (+ +) or above, the patient would be diagnosed as brucellosis. (5) Bacterial isolation: Brucella was isolated from blood, bone marrow, other body fluids, and excreta of patients. Any one of 1, 2, and 3 is suspected

TABLE 1 Follow-up status of patients with brucellosis in acute phase in three counties of Baotou city

	Number of people surveyed	Effective data	Effective rate
City			
Helin	24	14	58.3%
Damao	20	14	70.0%
Wuchuan	51	42	82.4%
Total	95	70	73.7%
Occupation			
Farmer	65	53	81.5%
Herdsman	17	10	58.8%
Meat and dairy processors	6	4	66.7%
Unemployed	7	3	42.9%
Total	95	70	73.7%

brucellosis. Suspected cases in accordance with any one of 4 and 5 are confirmed. The acute stage of brucellosis: The patient is within 6 months of onset has clinical symptoms and epidemiological contact history, and the test tube agglutination test is more than 1:100 + +. Serum samples of each confirmed patient were taken at 0 days, 15 days, 30 days, 60 days, 90 days, 180 days, and 360 days after diagnosis. The samples were subjected to RBPT, GTCA, and SAT analysis, and serum specific antibodies IgG and IgM were detected by ELISA. The detection reagents used for the Brucella RBPT, test tube agglutination test (SAT), and GICA were all qualified products and within the validity period.

RBPT: Divide the grid on dry slides, add 0.03 mL of serum sample on grid, then add 0.03 mL of Rose Bengal plate antigen, and observe continuously at room temperature for 5 min after mixing. If it is uniform pink with no agglutination, it is negative; if the serum is found to have agglutination reaction (liquid turbidity, particles or coagulation tablets), it can be judged as positive. GICA: A simple and rapid immunological detection technology established on the basis of immunofiltration technology. Principle: With NC membrane as the carrier, the capillary action of the microporous membrane is used to make the liquid dripped on one end of the membrane slowly permeate to the other end, just like chromatography.

SAT: Use 5 small test tubes to dilute the serum sample with sterile saline, the serum was diluted at 1:25, 1:50, 1:100, 1:200, and 1:400. Add the same amount of diluted antigen solution to the serum. After mixing, the serum dilution was 1:50, 1:100, 1:200, 1:400, and 1:800. At the same time, negative, positive, and antigen controls were made. Then, prepare the judgment turbidimetric tube. After all test tubes are fully mixed, incubate them at 37 ℃ for 20 ~22h, take them out, set them at room temperature for 2h, and take the turbidimetric tube as the standard to judge the results. Enzyme-linked immunosorbent assay (ELISA)[7]: The ELISA was used to test IgM and IgG. Universal binding 96-well microtiter plates (Thermo Fisher Scientific Oy, Vantaa, Finland) were coated with 0.5 μg tTG per well in 100 μl TBS-Ca buffer and incubated overnight (16 h) at 4°C. Thereafter, wells were washed five times with 300 μl TBS-TE buffer (10 mmol/l EDTA, 1 ml/l Tween 20,25 mmol/l Tris–HCl, 0.15 mol/l NaCl, and pH 7.4), and once with a solution of 50 g/l sucrose and 0.5 g/l thimerosal in TBS. Plates were sealed, dried at 20°C for 3 h, and kept at 4°C. Serum samples were diluted 1:100 in TBS-T buffer (25 mmol/l Tris–HCl, 0.15 mol/l NaCl, 1 ml/l Tween 20, and pH 7.4) and incubated in duplicate on tTG coated wells for 1 h. All incubations were performed at 20°C and washed with 300 μl TBS-T five times. The wells were incubated with dilutions of alkaline phosphatase-conjugated antibodies of rabbit anti-human IgG (Dako, Glostrup, Denmark), goat anti-human IgA, or goat anti-human IgM (Invitrogen Corporation, Carlsbad, USA) in TBS-T buffer for 30 min. The absorbances were read at 405 nm with 492 nm subtraction, and the antibody levels expressed in arbitrary units as percentages of the reference serum OD values. Results: The results were extrapolated from the absorption value of the standard curve established by the internal reference sample (high titer mixed serum), expressed in any unit per milliliter.

2.3 | Statistical processing

After collating and verifying the data using Excel 2013 software, a database was established and IBM SPSS23.0 statistics was used for analysis. The measurement data were expressed as mean ± standard deviation. Single-factor repeated measures' analysis of variance was used for comparisons in each test day. The counting data were expressed by constituent ratio and rate, and the row multiplication list chi-square test was used to for comparison between groups. The test level $\alpha = 0.05$, $p < 0.05$, the difference was considered to be statistically significant.

3 | RESULTS

3.1 | Basic information

Among the 70 patients with brucellosis in the acute phase that were effectively tracked, 49 were males, accounting for 70% of the total number, and 21 were females, accounting for 30% of the total number. The age was 54.0 ± 11.3 years, of which the oldest age was 81 years old and the youngest age was 23 years old. Most of the research objects are engaged in cattle and sheep breeding and livestock product processing.

3.2 | Serological testing of patients with brucellosis

3.2.1 | RBPT results

A total of 490 serum samples were collected from 70 effectively tracked patients at 0 days, 15 days, 30 days, 60 days, 90 days, 180 days, and 360 days after diagnosis. All patients were diagnosed with brucellosis, and the RBPT result was negative, and the negative result was defined as false negative. The RBPT test results showed that with the extension of the course of disease, the false-negative (–) results increased gradually, and the largest change was in the period of 30–60 days after diagnosis, with the constituent ratio increased by 12.9%; The number of strong positive (+ + +) and (+ + + +) results decreased gradually, and the largest change range of (+ + + +) was in the period of 0–15 days, and the constituent ratio decreased by 20.0%. The constituent ratios of negative and various positive were statistically significant at 0d, 15d, 30d, 60d, 90d, 180d, and 360d ($\chi^2 = 121.146$, $p = 0.0000$) (see Table 2).

3.2.2 | GICA results

All patients were diagnosed with brucellosis, the GICA result was negative, and the negative result was defined as false negative. The GICA results of 490 serum samples of patients with brucellosis in the acute stage showed that the false-negative results showed a fluctuating growth trend with the extension of the

TABLE 2 Comparison of RBPT results with different test days

Results		0d	15d	30d	60d	90d	180d	360d
-	n (%)	0(0%)	2(2.9%)	1(1.4%)	10(14.3%)	7(10.0%)	14(20.0%)	11(15.7%)
+	n (%)	4(5.7%)	9(12.9%)	25(35.7%)	19(27.1%)	19(27.1%)	17(24.3%)	21(30.0%)
++	n (%)	12(17.1%)	18(25.7%)	21(30.0%)	18(25.7%)	22(31.4%)	26(37.1%)	22(31.4%)
+++	n (%)	20(28.6%)	21(30.0%)	10(14.3%)	13(18.6%)	14(20.0%)	11(15.7%)	14(20.0%)
++++	n (%)	34(48.6%)	20(28.6%)	13(18.6%)	10(14.3%)	8(11.4%)	2(2.9%)	2(2.9%)
Total	n (%)	70(100%)	70(100%)	70(100%)	70(100%)	70(100%)	70(100%)	70(100%)

Note: Chi-square test: $\chi^2 = 121.146$, $p = 0.0000$

TABLE 3 Comparison of GICA test results with different test days

		0d	15d	30d	60d	90d	180d	360d
Negative	n (%)	3(4.3%)	5(7.1%)	3(4.3%)	9(12.9%)	3(4.3%)	14(20.0%)	15(21.4%)
Positive	n (%)	67(95.7%)	65(92.9%)	67(95.7%)	61(87.1%)	67(95.7%)	56(80.0%)	55(78.6%)
Total	n (%)	70(100%)	70(100%)	70(100%)	70(100%)	70(100%)	70(100%)	70(100%)

Note: Chi-square test: $\chi^2 = 25.257$, $p = 0.0003$.

TABLE 4 Comparison of SAT test results with different test days

Results		0d	15d	30d	60d	90d	180d	360d
1:100	n (%)	70(100.0%)	57(81.4%)	53(75.7%)	43(61.4%)	43(61.4%)	19(27.1%)	10(14.3%)
1:200	n (%)	54(77.1%)	37(52.9%)	35(50.0%)	24(34.3%)	25(35.7%)	10(14.3%)	5(7.1%)
1:400	n (%)	32(45.7%)	24(34.3%)	17(24.3%)	15(21.4%)	10(14.3%)	0(0.0%)	0(0.0%)
Total	n (%)	70(100%)	70(100%)	70(100%)	70(100%)	70(100%)	70(100%)	70(100%)

Note: Chi-square test for 1:100 test: $\chi^2 = 75.925$, $p = 0.0000$.

disease course. The constituent ratio of false-negative results was 4.3% on day 0 after diagnosis and 20.0% on day 180 after diagnosis. The constituent ratio of positive results decreased with the course of disease, but fluctuated in 60–90 days. The constituent ratios of negative and positive were statistically significant at 0d, 15d, 30d, 60d, 90d, 180d, and 360d ($\chi^2 = 25.257$, $p = 0.0003$) (See Table 3).

3.2.3 | SAT results

Serological SAT test is a standard method for diagnosing brucellosis and the criterion is 1:100 positive, but there are also false negatives. The results showed that with the extension of the course of disease, the positive results of 1:100 showed a stepwise decrease. The time range of the biggest change was 90–180 days, and the constituent ratio decreased by 34.3%. At 360 days, the positive results were only 14.3%. The constituent ratios of negative and positive in 1: 100 test were statistically significant at 0d, 15d, 30d, 60d, 90d, 180d, and 360d ($\chi^2 = 75.925$, $p = 0.0000$). The positive results of 1:200 and 1:400 showed the same trend. The ratio of 1:200 positive results within 360 days of diagnosis was only 7.1%, and after 180 days of diagnosis, there was no positive result at 1:400. (See Table 4).

3.2.4 | Antibody test results of patients with brucellosis

The antibody results showed that during the follow-up time, the IgG average value fluctuated with the extension of the course of the disease ($F = 26.381$, $p = 0.0131$). At 60 days after diagnosis, the IgG average value reached a maximum of 123.5, and at 180 days after diagnosis, it was a minimum of 89.8. During the follow-up period, the IgM average value showed a downward trend as the course of the disease prolonged ($F = 87.127$, $p = 0.0000$). In the two time periods of 15 to 30 days and 30 to 60 days after diagnosis, the decline was the largest, at 90 days, it fell to the lowest, and then, it increased slightly. (See Table 5, Figure 1).

3.3 | Symptom tracking of patients with brucellosis

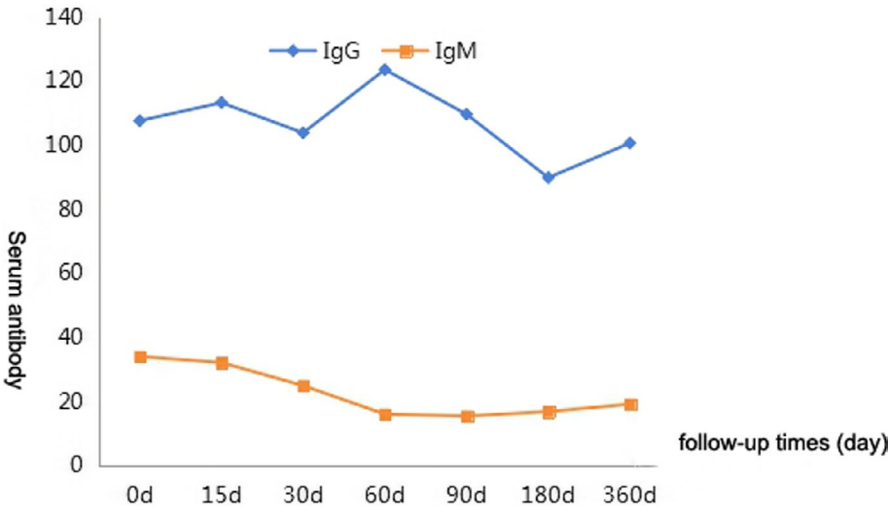
A total of 70 patients with brucellosis in the acute phase were effectively tracked. At the initial stage of onset (0d after diagnosis), the average number of brucellosis-related symptoms was 3.3, mainly joint muscle pain, fever, fatigue, and hyperhidrosis. On the day of diagnosis (day 0), the incidence of symptoms was more than 50%, and the incidence of joint and muscle pain was the highest, reaching 80%. With the extension of the course of disease, the average

TABLE 5 Comparison of IgG and IgM test results with different test days

Antibody	0d	15d	30d	60d	90d	180d	360d
IgG(ug/mL)	107.6	113.2	103.8	123.5	109.6	89.8	100.6
IgM(ug/mL)	34.0	32.1	24.9	16.0	15.4	16.8	19.1

Note: Single-factor repeated measures analysis of variance for IgG: $F = 26.381$, $p = 0.0131$. Single-factor repeated measures analysis of variance for IgM: $F = 87.127$, $p = 0.0000$.

FIGURE 1 Trend of antibody test results of serum samples with the course of the disease



number of symptoms dropped to 1.7 after 15 days and then gradually disappeared. (See Table 6).

4 | DISCUSSION

Brucellosis is found all over the world, especially in developing countries.[8] The highest incidence is in the Mediterranean Basin, the Arabian Peninsula, the Indian subcontinent, Mexico, and South and Central America.[9] Brucellosis was once quite serious in China and was basically controlled in the 1990s. However, at the beginning of the 21st century, with the development of animal husbandry, both human and animal morbidity showed a clear upward trend.[10] In recent years, the diagnosis of brucellosis has become a research hotspot. Especially in developing countries, a fast and accurate diagnostic method can not only diagnose correctly clinically, but is also a powerful measure to control the epidemic of brucellosis.[11] The gold standard for diagnosing Brucella infection is laboratory isolation and culture, but because the experiment is time-consuming and the biosafety risk is high, it is rarely used.[12,13] With the deepening of research on brucellosis, many improved traditional detection methods and new methods have emerged, such as the micro agglutination test (MAT),[14] fluorescence polarization test (FPA), and immunocapture agglutination technology (Brucellacapt). At present, the most commonly used serological testing methods in China are RBPT and SAT. Therefore, it is of great significance for the prevention and treatment of brucellosis to study the changes of serological test results and specific antibody titers of brucellosis patients with the course of disease.

In this article, 70 cases of brucellosis diagnosed in Helin County, Damao Banner, and Wuchuan County of Baotou City, Inner Mongolia Autonomous Region, were followed up for 360 days. It was found that the false-negative results of RBPT, GICA, and SAT in serological tests all increased with the extension of the disease course, and the false-negative rate after six months (180 days after diagnosis) were all higher than 20%. The RBPT test was used for initial screening. In the initial stage, that is, on the day of diagnosis, there are 0 false-negative cases, and 30 to 60 days after the diagnosis, the number of false-negative results has increased significantly; GICA was also a screening test, and on the day of diagnosis, there were 3 false-negative results. SAT1:100 positive was the basis for the diagnosis of brucellosis patients. On the day of diagnosis, the positive rate can reach 100%, and only 75.7% after one month. The above changes suggest that serological testing has high sensitivity in the initial stage of the disease. Patients should seek medical treatment within one month after the onset of suspected brucellosis symptoms, serum samples should be collected from patients for testing, and the accuracy rate is high. GICA has many advantages such as fast, sensitive, and convenient,[15] but its sensitivity is weaker than RBPT and SAT. Specific antibody detection and IgM level are beneficial to the early diagnosis of brucellosis, and IgG level is helpful to judge the stage of brucellosis.

The clinical symptoms of this disease are diverse: The heat type is wave-like fever, often feeling fatigue after a lot of sweating in the middle of the night or early morning, joint pain involves multiple joints, and is accompanied by symptoms of other systems. The natural course of untreated patients is 3–6 months (average 4 months), but it can be as short as 1 month or as long as several years. The course of the disease is divided into acute phase and chronic phase. The symptoms of acute phase are obvious, and the symptoms of chronic phase disappear gradually, so it is prone to missed diagnosis

TABLE 6 Statistical description of symptoms of brucellosis patients on different test days

Symptom		0d	15d	30d	60d	90d	180d	360d	0d
Fever	n (%)	48(69%)	4(6%)	2(3%)	1(1%)	1(1%)	0(0%)	0(0%)	48(69%)
Fatigue	n (%)	42(60%)	19(27%)	7(10%)	5(7%)	1(1%)	2(3%)	1(1%)	42(60%)
Hyperhidrosis	n (%)	45(64%)	10(14%)	7(10%)	8(11%)	1(1%)	0(0%)	0(0%)	45(64%)
Headache	n (%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)
Joint and muscle pain	n (%)	56(80%)	40(57%)	38(54%)	33(47%)	20(29%)	9(13%)	5(7%)	56(80%)
Nervous system symptoms	n (%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)
Genitourinary system symptoms	n (%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)
Enlarged liver and spleen lymph nodes	n (%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)
Average number of symptoms		3.3	1.7	1.2	0.8	0.4	0.3	0.2	3.3

and misdiagnosis. This is consistent with this study. Only 13% of patients still have joint and muscle pain six months after diagnosis (180d), and the other symptoms are not obvious or even disappear.

The limitations of this study are mainly as follows: First, this project mainly studies the change rule of serological test results with the course of disease, but does not analyze the serological test results and the type of brucellosis, as well as the gender, age, occupation, and other factors of patients, so the data mining is not sufficient; second, the pastoral area is difficult to follow up, it takes a lot of manpower, material, and financial resources, the lost follow-up rate is high, and the sample size is small. Further research is needed.

5 | CONCLUSIONS

The false-negative results of RBPT, GICA, and SAT increased with the prolongation of the course of disease, and the false-negative rate was higher than 20% after half a year. Patients infected with Brucella symptoms should be treated in time to avoid missed diagnosis and misdiagnosis due to too long duration. IgM level is conducive to the early diagnosis of brucellosis, and IgG level is helpful to judge the stage of brucellosis.

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Not applicable.

CONFLICTS OF INTEREST

All of the authors had no any personal, financial, commercial, or academicconflicts of interest separately.

AUTHOR CONTRIBUTIONS

TN and YR substantially contributed to the conception or design of the work. LH and ZW involved in acquisition, analysis, or interpretation of data for the work. SL, FM, and WY drafted the work or revised it critically for important intellectual content. All Authors finally approved the version to be published.

ETHICAL APPROVAL

This study was conducted in accordance with the Declaration of Helsinki and approved by Inner Mongolia Center for Disease Control and Research. All patients signed informed consent.

DATA AVAILABILITY STATEMENT

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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