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Original Research Article

Comparison of diagnostic accuracy of bactec culture, gene-xpert and histopathology in the diagnosis of genital tuberculosis in women with infertility

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ABSTRACT

Background: Female genital tuberculosis (FGTB) is often a silent disease sparing no age group but majority of patients are in the reproductive age. In infertility patient's incidence of FGTB varies from 3-16% in India but the actual incidence of genital tuberculosis may be under reported due to asymptomatic presentation and paucity of investigations.
Methods: Prospective case control study was conducted from June 2018 to May 2019 in LLRM Medical College Meerut. A total 100 Endometrial samples were collected during diagnostic laparoscopy from all suspected case of genital TB, presented with either primary or secondary infertility and samples sent for histopathology, Gene-xpert and Bactec culture.
Results: Out of 100 samples Bactec culture was positive in 2 samples, Gene-xpert positive in 3 samples. On histopathology out of 100 cases, non-specific endometritis was found in 1 case, tubercular-endometritis in 1 case, proliferative enometrium (anovulatory) in 40 cases and secretory endometrium found in 58 cases.
Conclusions: Female genital TB poses a diagnostic dilemma because of its varied presentation and lack of sensitive and specific method of diagnosis. Culture though remains the gold standard of diagnosis of female genital TB, gene-xpert, histopathology, Bactec culture or laparoscopy can be used for starting treatment. Endometrial biopsy on histopathology shows not only Tubercular endometritis but also gives hormone response on endometrium, local factors of endometrium concerning non-specific and specific infections and anovulatory cycles.

Keywords: Bactec culture, Gene-xpert, Histopathology, Infertility, Laparoscopy

INTRODUCTION

Infertility is defined as inability to conceive even after one year of unprotected sexual intercourse. There are various causes that result in infertility out of which genital tuberculosis is one of the important causes of infertility, especially in developing countries including India. Genital tuberculosis is a chronic disease and is almost always secondary and often has low grade symptomatology with very few specific complaints. It is estimated that 11% of patients lack symptoms.[1] The fallopian tubes are the initial site of involvement being affected in 100% cases of genital tuberculosis, followed by endometrium (50-60%), ovaries (20-30%), cervix (5-15%), vulva and vagina (1%). Most cases of confirmed genital tuberculosis will have a perfectly normal clinical examination, while a quarter of cases will present with an adenexal mass.[2]

Diagnosis of GT in early stage is very difficult. Early diagnosis may be associated with a favourable result before extensive genital damage occurs. Common presenting symptoms are nonspecific; hence diagnosis is difficult and elusive as affected patients have normal

serological tests like hemogram including TLC and DLC, ESR, Mantoux, normal chest x-ray. Imaging by abdominal and pelvic ultrasonography or abdominal and pelvic CT scan is often very nonspecific. Findings on imaging have been suggested to be helpful but final diagnosis is revealed by culture and histology.

Diagnostic laparoscopy may aid in early diagnosis and safe management of genital tuberculosis, preventing unnecessary laparotomies. The accuracy of this modality in diagnosing genital tuberculosis is not proven though.

The various test so far used in diagnosing genital tuberculosis are varied. Although, apart from mycobacterium TB in culture, none of them are 100%. Newer tests for the diagnosis of genital TB includes TB PCR, Bactec culture, Gene-xpert, QuantiFERON TB gold, QuantiFERON TB gold plus etc.

Bactec culture is a rapid radiometric culture and is based on measurement of carbon dioxide released by bacteria during growth in liquid medium. Radioactive carbon labelled substrate like palmitic acid or formic acid is used as marker for bacterial growth. It has higher sensitivity 80-90% and higher detection rate 7-10% and gives quicker result (5-10 days) and is also useful for drug susceptibility. The only disadvantage of this test is its cost. In this test homogenized sample is cultured using Bactec MGIT 960 system. 500 microlit. of sample is inoculated in MGIT tube containing 0.8 ml PANTA antibiotics and growth supplements. MGIT tubes are incubated in Bactec 960 instruments. This system automatically identifies positive sample.[3]

Gene-xpert is a cartridge based nucleic acid amplification test (CBNAAT) for simultaneous rapid tuberculosis diagnosis and rapid antibiotic sensitivity test and require less than 2 hours for result. It is an automated diagnostic test that can identify MTB-DNA and resistance to Rifampicin. It has specificity of 99%. In this test 1ml of homogenized sample is added to 2 ml of Gene-xpert sample reagent. Mixture is vortexed for 30 seconds. The sample is left to stand for 15 minutes at room temperature and then 2 ml of mixture sample is transferred to the test cartridge. Cartridge is loaded onto the xpert instrument. Results will be reported as positive or negative and sensitivity by RIF resistance determining region of the rpoB gene with molecular beacons within 2 hours.[4]

The objective of this study was to compare Bactec, Gene-xpert and HPE in the diagnosis of genital tuberculosis in infertility patients.

METHODS

This study was conducted in department of obstetrics and gynecology, in collaboration with department of pathology and department of microbiology in S.V.B.P. Hospital L.L.R.M. Medical College Meerut from June 2018 to May 2019.

This was a prospective case control study including 100 patients who underwent diagnostic laparoscopy for infertility. Clearance was obtained from Ethical committee of hospital before starting the study. In this study total of 100 patients were enrolled who presented with Primary or Secondary infertility. After fulfilling inclusion and exclusion criteria, they were recruited to undergo Diagnostic Laparoscopy and hysteroscopy. Each patient was explained regarding the procedure and proper written consent was taken after thorough history, examination and investigations. Endometrial samples were collected from every patients and were subjected to further testing.

Inclusion criteria

- Women with primary or secondary infertility suspected for genital tuberculosis
- Infertile women with normal husband semen analysis.

Exclusion criteria

- Women with HIV positive status
- Fibroid distorting the endometrial cavity
- Congenital abnormalities of uterus
- Cervical polyp and malignancy.

All suspected cases of genital Tuberculosis presenting with primary and secondary infertility were included in this study. Known cases of malignancy and pregnancy excluded from study. Detailed history, general physical and gynaecological examination followed by baseline investigations. All patients underwent diagnostic laparoscopy under general anaesthesia preferable in premenstrual phase (D21-D28) and were evaluated for suggestive findings of genital tuberculosis and endometrial biopsy taken. Samples were divided in three parts. First two samples kept in normal saline send for Bactec culture and Gene-xpert. Third sample in 10% formalin for histopathology. Patients profile, history, investigations, laparoscopic findings, tests ordered and finally their result were recorded in proforma and analyzed.

Statistical analysis

Descriptive statistics was used to explain the data using Microsoft excel and MedCalc software.

RESULTS

Demographic profile of patients

Age of the patients

In this study, the youngest patients were of 23 years and eldest was of 34 years of age. The mean age of the patients was 27.94. Majority of the patients in this study was between 26-30 years (51%).

Type of infertility

Out of 100 patients, 69 (69%) patients had primary infertility and 31 (31%) patients had secondary infertility (Table 1).

Table 1: Distribution of type of infertility.

Type of infertility	No. of patients	Percentage
Primary	69	69%
Secondary	31	31%
Total	100	100%

Table 2: Maximum and minimum duration of infertility.

Minimum	Maximum	Range	Mean	SD
3 years	15 years	12 years	6.35	2.1648

Table 3: Distribution of duration of infertility.

Duration of infertility	No. of patients	Percentage
1-5 years	34	34%
6-10 years	60	60%
>10 years	6	6%
Total	100	100%

Duration of infertility

The overall mean duration of infertility among the patients ranges from minimum 3 years to maximum 15 years. However more than half of the patients had infertility of 6-10 years. The overall mean duration of infertility was 6.35 years (Table 2 and 3).

Table 4: Distribution according to history of TB in patients or in family.

	Percentage	Absent
Family history of TB	11	89
Past history of TB	19	81

Table 5: Distribution of patients according to laparoscopic findings.

Laparoscopic findings	Frequency	Percentage
Normal	61	61%
Findings s/o FGTB	39	39%
Total	100	100%

Obstetric history

- *Parity and abortions*

Out of total 100 patients 69 patients were nulliparous and 5 patients were para1 and 26 patients had history of abortion. Out of 26 patients with abortion 22 had history of one abortion and 4 patients had history of two abortions.

Different menstrual patterns

Majority of women had normal menstrual function i.e., 71, oligomenorrhoea in 16 patients, hypomenorrhea in 6 patients, 6 patients had menorrhagia and 1 patient had secondary amenorrhoea.

History of tuberculosis in patients or in family

Out of total 100 patients 11 had family history of tuberculosis and 19 had past history of tuberculosis (Table 4).

Distribution on the basis of ESR

Out of total 100 patients 16 patients had raised ESR and 84 patients had normal ESR.

Distribution of patients according to Mantoux

Induration more than 10mm indicate Positive reaction between 48-72 hours. Out of total 100 patients, 21 patients had positive result and 79 had negative result.

Out of 21 Mantoux positive patients, 15 had positive findings s/o genital tuberculosis on laparoscopy and 6 had normal laparoscopic findings.

Distribution of patients according to laparoscopic findings

Although laparoscopy is an invasive procedure, but it is an important tool in the evaluation of infertility and in the diagnosis of various pelvic conditions. With the help of laparoscope ovaries, fallopian tubes, peritoneal cavity, uterine surface etc inspected for stigmata of genital tuberculosis, that aids in the diagnosis of genital tuberculosis. The laparoscopic findings suggestive of genital tuberculosis may vary from normal appearance to tubercles on the surface, fimbrial block, fimbrial phimosis, tubal beading, peritubal adhesions, periovarian adhesions, tub ovarian mass, hydrosalpinx and rigid tubes etc.

In the present study, laparoscopic findings suggested that 61% patients of the study patients had normal findings and 39% patients had laparoscopic features suggestive of genital tuberculosis (Table 5 and 6).

Peritoneum

On laparoscopy 67% patients had normal peritoneal findings. While tubercle, peritoneal inclusion cysts and adhesions were found in 6%, 8% and 19% respectively.

Table 6: Distribution of patients according to various laparoscopic findings.

Laparoscopy	Findings	Frequency	Percentage
Peritoneum	Normal	67	67%
	Tubercles	6	6%
	Adhesions	19	19%
	Peritoneal inclusion cyst	8	8%
	Total	100	100%
Uterus	Normal in shape and contour	90	90%
	Tubercles	4	4%
	Increased vascularity	6	6%
	Total	100	100%
Ovaries	Normal	62	62%
	Bulky/PCOD	11	11%
	Functional Ovarian cyst	16	16%
	Peri ovarian adhesions	6	6%
	Endometriotic cyst	5	5%
	Total	100	100%
Fallopian tube	Normal in appearance	63	63%
	Unilateral hydrosalpinx	3	3%
	Bilateral hydrosalpinx	4	4%
	Tubercles	4	4%
	Peritubal adhesions	15	15%
	Bilateral tubo-ovarian mass	6	6%
	Beaded appearance	5	5%
	Total	100	100%
	Perihepatic adhesions	2	2%
	Bowel and omental adhesions	3	3%
Chromopertubation	Bilateral spill of dye	66	66%
	Unilateral spill of dye	9	9%
	Absent spill of dye	19	19%
	Loculated spill of dye	6	6%
	Total	100	100%

Uterus

Out of total, 90% patients had normal uterine findings while 4% patients had tubercles and 6% had increased vascularity.

Ovaries

A total 62% patients had normal looking ovaries while 5%, 6%, 11% and 16% patients had endometrial cysts, peri-ovarian adhesions, bulky ovaries and functional ovarian cysts respectively.

Fallopian tube

A total 63% patients had normal appearing fallopian tubes while 3%, 4%, 4%, 5%, 6% and 15% patients had unilateral hyrosalpinx, bilateral hydrosalpinx, tubercles, beaded tubes, T-O mass and peritubal adhesions respectively.

A total 2% patients had perihepatic adhesions and 3% patients had bowel and omental adhesions.

Table 7: Distribution of patients according to hysteroscopic findings.

Hysteroscopic findings	Frequency	Percentage
Normal	88	88%
Findings s/o FGTB	12	12%
Total	100	100%

Chromopertubation

On chromopertubation 66% patients had bilateral spill of dye, 6% patients had loculated spill of dye, 9% had unilateral spill of dye and 19% patients had absent spill of dye.

Distribution of patients according to hysteroscopic findings

On hysteroscopy out of total 100 patients 88 patients had normal study, 4 patients had bald endometrium, 5 patients had distorted ostium and 3 patients had synechiae (Table 7 and 8).

Distribution of samples according to Bactec culture

Out of 100 samples Bactec was positive in 2 samples (2%), both had also findings s/o genital tuberculosis on laparoscopy (Table 9 and 10).

Table 8: Distribution of patients according to various hysteroscopic findings suggesting FGTB.

Findings	Frequency	Percentage
Bald endometrium	4	4%
Distorted ostium	5	5%
Synechiae	3	3%
Normal	88	88%
Total	100	100%

Table 9: Distribution of samples according to Bactec culture.

	Total samples	No. of positive sample for Bactec	No. of negative sample	Percentage
Laparoscopic finding s/o FGTB	39	2	37	5.1%
Normal laparoscopic findings	61	0	61	0%
Total	100	2	98	

Table 10: Distribution according to Bactec culture.

	Sensitivity	Specificity	PPV	NPV	p value
Bactec	5.12	100	100	62.24	0.0739

Table 11: Distribution of samples according to Gene-xpert.

	Total samples	No. of positive samples	No. of negative samples	Positive samples percentage
Laparoscopic findings s/o FGTB	39	2	37	5.10%
Normal laparoscopic findings	61	1	60	1.63%

Table 12: Distribution of samples according to Gene-xpert.

	Sensitivity	Specificity	PPV	NPV	p value
Gene-xpert	5.128	98.36	66.66	61.85	0.318

Table 13: Distribution of samples according to HPE.

	Total	No. of positive samples	No. of negative samples	Positive sample percentage
Laparoscopic findings s/o FGTB	39	1	38	2.56%
Normal on laparoscopy	61	0	61	00%

Distribution of samples according to Gene-xpert

Out of total 100 samples Gene-xpert was positive in 3 samples. Out of 3, 2 had findings s/o genital tuberculosis and 1 had normal finding on laparoscopy and none of them were Rifampicin resistant (Table 11 and 12).

Distribution of samples according to HPE (histopathology)

On histopathological examination of endometrium, 1 case was positive for tubercular endometritis, non-specific endometritis in 1 case, proliferative endometrium (anovulatory) was found in 40 cases and secretory endometrium was found in 58 cases (Table 13, 14 and 15).

Table 14: Distribution of different histopathological pattern of samples.

Histopathology	Frequency	Percentage
Tubercular endometritis	1	1%
Non-specific endometritis	1	1%
Proliferative phase	40	40%
Secretory phase	58	58%
Total	100	100%

Table 15: Distribution of HPE samples.

	Sensitivity	Specificity	PPV	NPV	p value
HPE	2.56	100	100	61.61	0.20

Table 16: Comparison of diagnostic yield of various tests.

Tests	Sensitivity	Specificity	PPV	NPP	p value
Bactec	5.12%	100%	100%	62.24%	0.0739
Gene-xpert	5.12%	98.36%	66.66%	61.85%	0.318
HPE	2.56%	100%	100%	61.61%	0.20

DISCUSSION

FGTB has become a challenging disease both from diagnostic and therapeutic point of view as it has very few characteristic symptoms. On the basis of clinical presentations, a woman cannot be diagnosed with FGTB.

The diagnostic dilemma arises because of the varied clinical presentation of the disease confounded by diverse results on imaging, laparoscopy, histopathology and mixed bag of bacteriological and serological tests, each of which has its limitation in diagnostic sensitivity and specificity (Table 16).

Table 17: Comparison of type of infertility in various studies.

Author	Year	Primary infertility	Secondary infertility
Kiran et al	2017	72%	28%
Jindal N et al	2017	76%	24%
Mahesweta et al	2017	74.3%	25.7%
Danish et al	2018	60.6%	39.4%
Roopakshi et al	2018	46.9%	53.1%
Chesta et al	2018	74%	26%
Nikita et al	2019	67%	33%
Present study	2019	69%	31%

Table 18: Comparison of history of tuberculosis.

Author	Year	H/o of TB
Nidhi et al	2017	6.29%
Roopakshi et al	2018	11.2% (family h/o TB) 23.5% (past h/o TB)
Nikita et al	2019	14.28%
Present study	2019	11% (family h/o TB) 19% (past h/o TB)

If patients are adequately treated before their tubes are irreversibly damaged, the chances of successful pregnancy are reasonably good therefore early diagnosis of genital tuberculosis is needed for better outcome.

Hystero-laparoscopy are useful to diagnose the FGTB by presence of adhesions, presence of tubercles etc., that can help to sort the highly suspicious FGTB patients.

Table 19: Comparison of percentage of laparoscopic findings.

Author	Year	Normal findings	Abnormal findings (s/o GTB)
Mala YM et al	2017	73.3%	26.6%
Thangappah et a	2018	53.8%	46.2%
Nikita et al	2019	72%	28%
Diksha et al	2019	60.3%	39.7%
Present study	2019	61%	39%

Table 20: Comparison of BACTEC positivity in various studies.

Author	Year	Bactec positive (%)
Geetika et al	2013	8.8%
Riden et al	2017	27%
Mahesweta et al	2017	2.85%
Present study	2019	2%

Table 21: Comparison of Gene-xpert positivity in different studies.

Author	Year	Gene-xpert positive (%)
Riden et al	2017	1.6%
Roopakshi et al	2018	0%
Danish et al	2019	7.25%
Diksha et al	2019	5%
Present study	2019	3%

A total 100 infertile patients suspected of genital tuberculosis were included in this study out of which majority of patients (69%) had primary infertility and 31% patients had secondary infertility (Table 17). Most of the women were in age group of 26-30 years (51%) and mean age of the patients was 27.94 years with mean duration of infertility 6.35 years. Out of total 100 patients 11 had family h/o tuberculosis and 19 patients had h/o pulmonary tuberculosis in past (Table 18). A study done by Nikita et al in which 14.28% patients had history of tuberculosis.[5]

In this study majority of patients had normal menstrual pattern (71%) while oligomenorrhoea in 16%, hypomenorrhea in 6%, menorrhagia in 6% and secondary

amenorrhea in 1% patients. Comparable study done by Mahesweta et al in 2017.[6]

Table 22: Comparison of percentage of endometrial tuberculosis on HPE.

Author	Year	HPE S/O FGTB (%)
Geetika et al	2013	2.6%
Srivastava et al	2014	1.3%
Mahesweta et al	2017	0%
Roopakshi et al	2018	1.23%
Thangappah	2018	4%
Nikita et al	2019	7.14%
Danish et al	2019	0%
Present study	2019	1%

Out of 100 patients 16 patients had raised ESR and 84 patients had normal ESR and out of 100 patients 21 patients had positive result (>10 mm) and 79 had negative Mantoux test.

In the present study, laparoscopic findings suggested that 61% patients of the study patients had normal findings and 39% patients had laparoscopic features suggestive of genital tuberculosis. Various laparoscopic findings shown in Table 6.

Comparable study done by Diksha et al in which 60.3% patients had normal laparoscopic findings and 39.7% patients had laparoscopic findings suggestive of genital tuberculosis like delayed dye spillage 6 (8.8%), hydrosalpinx present 7 (10.3%), beaded appearance of fallopian tubes 5 (7.3%), granulomas 1 (1.4%), pelvic congestion 1 (1.4%), pelvic adhesions 5 (7.3%) and bilateral dye spillage absent 15 (22%) and unilateral dye spillage seen 10 (14.7%).[7]

This study results are comparable with various studies done in past (Table 19).

Hysteroscopy

On hysteroscopic visualization of the uterine cavity in genital TB may show normal cavity with bi lateral open ostia in early stage.

In this study on hysteroscopy out of Total 100 patients 88 patients (88%) had normal study,4 patients had bald endometrium, 5 patients had distorted ostium and 3 patients had synechiae.

Comparable study done by Mahesweta et al in which on hysteroscopy, 29 cases (82.9%) of the patients had normal findings and 3 cases had hysteroscopic features suggestive of GTB.[8] Bald endometrium, distorted ostium, synechiae was present in 12.5%, 12.5%, 12.5% cases respectively. In the study conducted by Ashabaxi et al, the intrauterine synechie was observed only in 3.57% and fibrosed ostia in 7.14%.

Bactec culture

It is a type of liquid culture and automatically identifies positive samples. In this study out of 100 samples Bactec was positive in 2 samples (2%). Both Bactec positive sample also had laparoscopic findings suggestive of FGTB. On the basis of this sensitivity, specificity, positive predictive value and negative predictive value was 5.26%, 100%, 100% and 62.24% respectively. Comparable study done by Mahesweta et al, in which TB Bactec was positive in 2.85% (n = 1), and none of the cases had histopathological evidence of genital TB.

Various studies of percentage of Bactec positivity are compared which were done in past with this study (Table 20).

Gene-xpert

Gene-xpert is a cartridge based nucleic acid amplification test (CBNAAT) for simultaneous rapid tuberculosis diagnosis and rapid antibiotic sensitivity test, (Less than 2 hours). It is an automated diagnostic test that can be identify MTB-DNA and resistance to Rifampicin. WHO endorsed the Gene-xpert for MTB diagnosis in endemic countries in December 2010. It has specificity of 99%. In this study out of total 100 samples Gene-xpert was positive in 3 samples (3%). Out of 3 positive result containing patients 2 had laparoscopic findings suggestive of FGTB and 1 had normal laparoscopic findings. On the basis of this sensitivity, specificity, positive predictive value and negative predictive value was 5.26%, 98.36%, 66.66% and 61.85% respectively. Comparable study done by Riden et al in which Gene-xpert positive in 1.6% patients.[9] Various studies of Gene-xpert positivity are compared (Table 21).

HPE (histopathological evaluation)

Demonstration of granuloma with or without Langerhans giant cell on histopathology is diagnostic of genital TB. In this study on histopathological examination of endometrium, 1 case (1%) was positive for tubercular endometritis, non-specific endometritis in 1 case (1%), proliferative endometrium (anovulatory) was found in 40 cases (40%) and secretory endometrium was found in 58 cases (58%).

One positive result having tubercular endometritis also had laparoscopic finding suggestive of FGTB as well as positive for Bactec and Gene-xpert. On the basis of this sensitivity, specificity, positive predictive value and negative predictive value was 2.56%, 100%, 100% and 61.61% respectively. Comparable study done by Roopakshi et al, in which on histopathological examination of endometrium, 1 case (1.23%) was found positive for tubercular endometritis, proliferative endometrium (anovulatory) was found in 34 cases (41.9%), non-specific endometritis in 1 case (1.23%) and secretory endometrium was found in 45 cases (55.55%).

Various studies are compared on the basis of percentage of endometrial tuberculosis on HPE Table.[22]

CONCLUSION

Female genital tuberculosis (FGTB) has become a challenging disease from diagnostic point of view despite of availability of potent drugs for treatment as it has very few characteristic symptoms. The diagnosis of genital tuberculosis is seldom suggested from history or physical examinations. The diagnostic dilemma arises because of the varied presentation of the disease confounded by diverse results on imaging, laparoscopy, histopathology and mixed bag of bacteriological and serological tests, each of which has its limitation in diagnostic sensitivity and specificity. If the patients are adequately treated before their tubes are irreversibly damaged, the chances of successful pregnancy are reasonably good. Although laparoscopy is an invasive procedure, it aids in visual inspection of ovaries, fallopian tubes, peritoneal cavity and biopsy of the tubercular lesions. The laparoscopic findings of genital TB may vary from normal appearance to tubercle on surface, fimbrial block, tubal beading, peritubal adhesions, T-O mass, hydrosalpinx etc. It aids in speedy diagnosis and therefore, early implementation of treatment and thereby preventing massive and sometimes irreparable damage to vital reproductive structures. Therefore, diagnostic laparoscopy is recommended in all clinically suspicious cases of genital TB.

Combination of HPE and microbiological tests like Bactec, Gene-xpert are important method for detection of genital TB. Although Bactec and Gene-xpert is said to have high sensitivity and high specificity but still there is limited role in the early diagnosis of genital TB from endometrial sample because of regular shedding of endometrial lining during each menstrual cycle.

Endometrial biopsy on Histopathology shows not only Tubercular endometritis but also gives hormone response on endometrium, local factors of endometrium concerning non-specific and specific infections and anovulatory cycles.

Diagnostic dilemma of genital tuberculosis still exist, therefore more larger case control studies are needed to find out more sensitive and specific tests to diagnose genital TB i.e. despite of availability of various techniques, diagnostic dilemma for genital TB still exist. Until date, none of the available tests can pick up all cases of genital tuberculosis.

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