Non-palpable breast lesions marked with coal suspension: evaluation of anatomopathological aspects, viability of interpretation and inflammatory response

Lesões impalpáveis da mama marcadas com suspensão de carvão: avaliação de aspectos anatomopatológicos, viabilidade de interpretação e resposta inflamatória

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A B S T R A C T

Objective: To analyze the effectiveness of the inert coal suspension in marking impalpable breast lesions and the morphological alterations associated with its use, and to determine whether there is impairment in the interpretation of these lesions by the pathologist. **Methods**: We treated 135 cases of impalpable breast lesions, previously marked with coal suspension. The slides stained with HE containing carbon pigments were analyzed by optical microscopy, with which we performed quantitative and qualitative assessments of the inflammatory response and determination of the presence or absence of damage to the pathological diagnosis. The indices quantitatively evaluated as for the inflammatory response were lymphocytes, neutrophils and giant cells counts, all observed and quantified in high-power fields. We also evaluated the amount and distribution of coal present in the lesions. **Results**: In quantitative and qualitative assessment of the inflammatory response, we observed "foreign body" granulomas in all samples regardless of the amount of coal. Regarding lymphocytic inflammatory response, 5.19% of the samples showed no lymphocytic infiltration; in 82.22% it was mild in intensity, and moderate in 12.59%. As to the acute inflammatory response, we observed absence of neutrophilic exudation in 42.96% of the specimens, discrete neutrophilic exudation in 42.22%, moderate in 11.11% and severe, in the form of microabscesses, in 3.7% of cases. In this series there were five lumps of coal, with paralesional position, which did not generate interference in the morphological analysis. **Conclusion**: This method is an effective marker of non-palpable lesions, has low cost, high effectiveness, is subject to histological analysis, besides being easy to perform; it is comfortable for the patient and a great help to the surgeon and pathologist on the location of these lesions.

Key words: Charcoal. Mophology. Breast. Neoplasms. Breast neoplasms.

INTRODUCTION

In recent decades, the rate of detection of impalpable breast lesions, also called subclinical, has grown considerably. The non-palpable lesions are characterized by being non-nodular or nodular, the latter usually with size less than 1cm, with the possibility of reaching larger sizes depending on the characteristics of the parenchyma and size of the affected breast.

The size of non-palpable invasive breast tumors is, on average, of 10mm. Among non-palpable breast lesions, the ones causing most interest are those suspicious of malignancy. They appear as nodules or even take other radiographic features, such as calcifications[1,2], abnormalities in soft tissue, architectural distortion and the presence of irregular masses.

The high detection rate is due to the implementation of prevention programs, increased participation of mammographic screening and marked improvement in imaging techniques, favoring the finding of changes, more refined and smaller everyday[1-4]. Until now mammography is considered the most specific and

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sensitive exam for the diagnosis of breast cancer in its earliest form[2].

More recently MRI has assumed an important role in the diagnosis of early lesions, but even with high sensitivity this method has low specificity. Likewise, the use of ultrasonography is recommended as a complementary method to mammography and MRI, thus becoming an important tool, especially in cases of small, dense breasts[3].

The annual mammographic screening in women over 40 years detects approximately 100 to 200 new cases of suspicious lesions, which are presented as non-palpable in every 20,000 mammograms. These lesions require histological study, one of the options being its preoperative location and marking[1,5-13].

Despite the good performance of mammography in the identification of early-stage breast cancer, 10% to 30% of non-palpable lesions are malignant when biopsied[14], and 15% to 25% are intraductal lesions[5]. Consequently, the correct location and subsequent excision of non-palpable lesions that are malignant are determinants to surgical success. There is need for previous marking of the lesion site, so that it can be correctly identified by the surgeon, accurately and appropriately removed, taking into consideration the cosmetic results arising from the removal of the least amount of tissue.

Additionally, it is of great importance to provide the pathologist with ideal conditions for the handling of the specimen, macroscopic and microscopic identification of lesions and their interpretation, redounding in an accurate morphological diagnosis, both qualitatively and quantitatively.

The objectives of this study were to evaluate the effectiveness and feasibility of marking of non-palpable breast lesions with 4% inert aqueous carbon; to evaluate whether the solution of coal hampers or not the interpretation of histopathological diagnosis; to quantify the inflammatory response in the presence of coal; and to check the pattern of distribution of coal in relation to the target lesions.

METHODS

This study was conducted in CITOLAB – Laboratory of Cytology, Molecular Biology and Pathology, in Curitiba, Paraná State – PR, Brazil.

The initial sample consisted of 135 surgical specimens originated from 109 patients with impalpable breast lesions marked by mammography or ultrasound puncture and injection of coal suspension. Each lesion was submitted to prior review for selection of slides containing the coal pigment and, among these, those that had the most significant morphological changes in relation to inflammatory components and the presence of coal. The specimens were identified and processed according to routine histological techniques, being previously stored and fixed in 10% formalin.

The samples were evaluated in the totality of their slides. We selected those with changes associated with the presence of coal and with the impalpable alterations that prompted surgery. We then identified the paraffin blocks the corresponding containing lesions and subjected them to microtomy for new 4mm-thick slices. They were subsequently stained with hematoxylin and eosin (HE) and reassessed. The 135 samples were from 109 female patients. Of these, 89 patients had only one lesion in the investigated and coal-labeled breast; 15 had two lesions; four, three lesions; and one, four lesions. Their ages ranged between 21 and 87 years, with an average of 48 years.

The microscope slides used for analysis were coupled to a photomicrograph system. All selected specimens were first visualized with 100 increase (10x) lens, so the coal impregnation area could be located, as well as to attest the presence of the "target" lesion from the surgical procedure. Next, the analysis concentrated on the region of highest intensity of inflammatory reaction associated with the presence of coal, 400 increases (40x) lens being used. In all cases ten magnified fields were evaluated, with differential count in the number of lymphocytes, neutrophils and multinucleated macrophages. Numerical data were standardized according to the number and distribution of inflammatory monomorphonuclear and polymorphonuclear elements, as well as the amount of vascularity present in the sample, according to parameters specified below.

The morphological criteria and numerization for acute inflammation were based on vascular proliferation (VP) and number of polymorphonuclears (PMN) as follows: absent (0); discreet, isolated VP and PMN (1); moderate, VP and PMN aggregates in part of the affected area (2); pronounced, confluent and aggregated VP and PMN throughout the affected area (3).

For chronic inflammation we quantified lymphocytes / plasma cells and macrophages (MMN), as follows: absent, without MMN; discrete, isolated MMN; moderate, MMN aggregates in part of the lesion; pronounced, aggregated and confluent MMN throughout the lesion.

Acute inflammation was considered as the summ: 0 = no acute inflammation; 1 or 2 = discrete acute inflammation; 3 or 4 = moderate acute inflammation; 5 or 6 = severe acute inflammation.

The distribution of coal represented the amount of coal found according to its introduction by needle in the lesion and its surrounding during the injection of the suspension in the breast. We evaluated the distribution pattern of the presence of coal in relation to the analyzed lesion and rated it as intralesional, perilesional, paralesional or a combination of two or more of these distributions.

We also verified the presence or absence of a lesion in the specimen, prepared new diagnostic assessment and acknowledged any interference in the quality of

morphological analysis and the final pathological diagnosis caused by the presence of coal, graduating the difficulty of interpretation of the target lesion in a numerical scale from zero to ten.

RESULTS

All parameters analyzed and results were imputed in the database in the form of a spreadsheets.

We analyzed 135 surgical specimens obtained from excision of impalpable breast lesions in 109 patients, 82 located in the left breast and 53 in the right. Among the samples, 88 were represented by pure nodes, eight nodes associated with microcalcifications, one was represented by an impalpable plate/mass and 38 cases were investigated solely due to the presence of microcalcifications.

Of the 135 cases analyzed, 35 were malignant lesions (26.66%), 97 benign lesions (71.85%), one case consisted of malignancy associated with benign lesions (0.74%), and in two specimens (1.48%) no lesion was observed (Table 1 and Figure 1).

The smaller diameter of nodular lesions was 0.3 cm, the greatest 2cm. The average volume observed in nodular pattern lesions was 0.60 $cm^{3,}$ varying between 0.02 and 2.3 cm^3.

The distribution of coal in relation to the target lesion was intralesional in eight cases (5.92%) and paralesional in 61 (45.14%) (Figure 2); perilesional in 26 cases (19, 26%), intra and perilesional in 21 (15.50%), intra and paralesional in 11 (8.14%) peri and paralesional in five (3.70%) and intra, peri and paralesional in one case (0.74%) (Figure 3).

Regarding the chronic inflammatory process, there were multinucleated macrophages with intracytoplasmic coal particles in all cases (100%). With respect to lymphocyte component, 111 cases (82.22%) showed mild

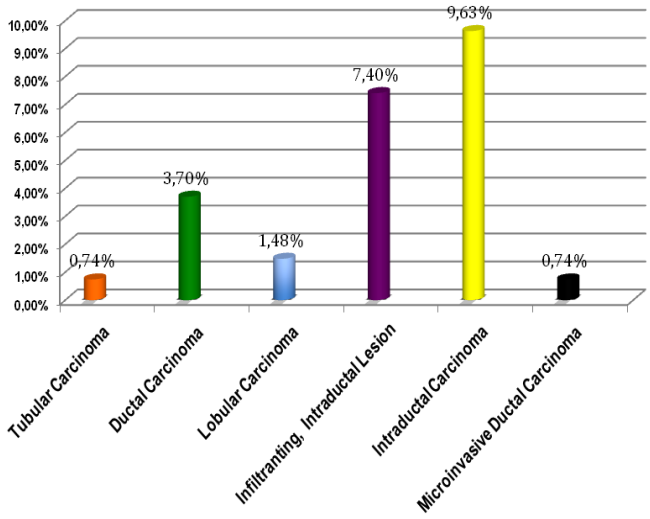

Figure 1 - *Distribution of malignant lesions.*

Table 1 - Type of histological lesions investigated.

Histological Lesion	Diagnoses anatomopathological n=	%
Fibroadenoma	51	37.78
Papiloma	5	3.70
Fibrous/ sclerotic plaque	4	2.96
Fat necrosis	1	0.74
Intramammary lymph node	1	0.74
Hamartoma	4	2.97
Leiomyoma	1	0.74
Adenomyoepithelioma	2	1.48
Nonproliferative fibrocystic condition	1	0.74
Proliferative fibrocystic condition	26	19.26
Sclerosing radiated lesion	3	2.23
Intraductal carcinoma associated with fibroadenoma	1	0.74
Intraductal carcinoma	12	8.89
Lobular neoplasia "in situ"	1	0.74
Infiltrating ductal carcinoma	5	3.70
Infiltrating ductal carcinoma + Ductal carcinoma "in situ"	10	7.40
Infiltrating lobular carcinoma	2	1.48
Infiltrating lobular carcinoma + ductal carcinoma "in situ"	1	0.74
Microinvasive ductal carcinoma	1	0.74
Tubular carcinoma	1	0.74
No histological lesions	2	1.48

intensity, 17 moderate (12.59%) and seven (5.19%) had no lymphocytes (Figure 4).

The acute inflammatory response, on its turn, was not observed in 58 (42.96%) cases, was mild in 57 (42.23%), moderate in 15 (11.11%) and severe, with abscess formation in five cases (3.7%) (Figure 5).

The amount of carbon present in the samples was mild in 39 cases (29%), moderate in 91 (67.4%) and severe, with black nodules, in five (3.7%).

The amount of coal suspension in the specimens was mild in 29%, moderate in 67.4% and severe in 3.7%.

In two of the surgical specimens (1.48%) we could not detect the histological lesions, and in one the amount of coal observed was minimal.

In the surroundings of lesions from 12 cases we observed fat necrosis, and in one case, besides necrosis, displacement artifacts that imposed an immunohistochemical study for defining infiltrating malignant lesion or *in situ* lesion.

In all cases containing detectable lesions (98.52%) there was no impairment to histological analysis and the final pathological diagnosis was straight, with zero difficulty level, taking into consideration the scale from zero to ten.

DISCUSSION

The increasing use of mammography and ultrasound in screening programs for breast diseases, associated with technological devices generating image with greater accuracy, has enabled the finding of increasing number of non-palpable breast lesions[15].

The adequate preoperative localization is of great importance, so the target lesion is successfully removed, with excision of smaller amount of normal breast tissue, following the trend of currently recommended conservative operations. Several methods have been proposed and different techniques, from the "x" marking with single and multiple needles[4,5,7,10], metallic wire[5,16], dye injection[1,17-19], coal suspension[1,3,4,6,11,20] and the use of radioisotopes[21,22].

The mostly used method throughout the world today is the needle / metallic wire location. It is a technique that allows adequate preoperative marking of 92-98% of palpable breast lesions. However, it is associated with complications and failure rates, which, according to the

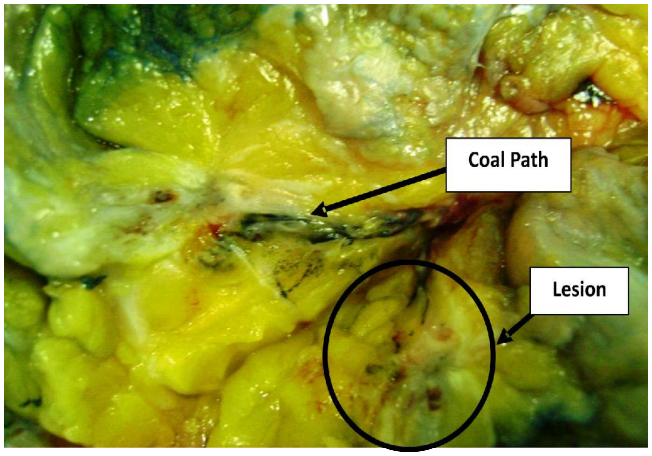

Figure 2 - *Macroscopy of paralesional coal suspension.*

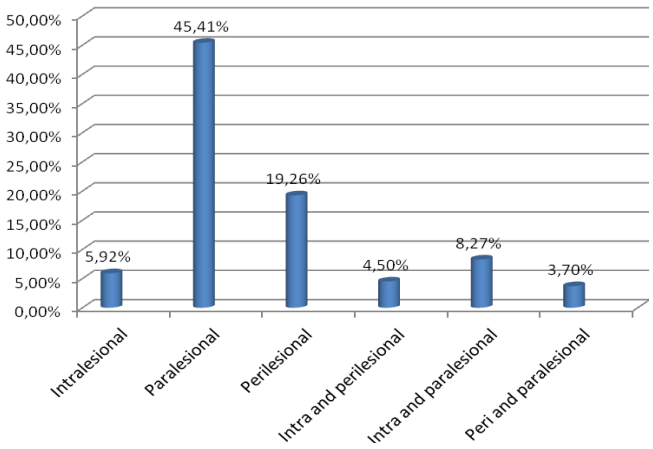

Figure 3 - *Distribution of the coal suspension in relation to the lesion.*

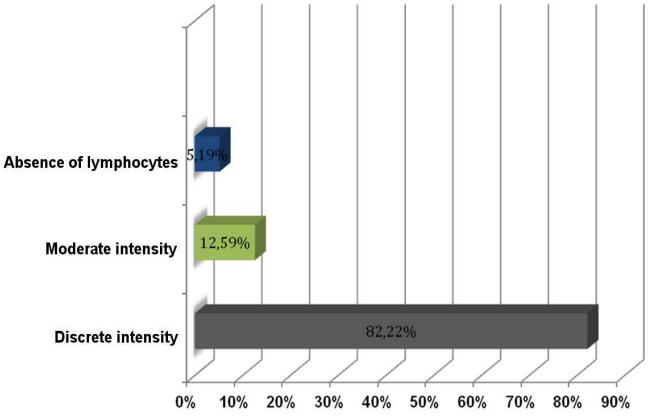

Figure 4 - *Chronic inflammation -% lymphocytes.*

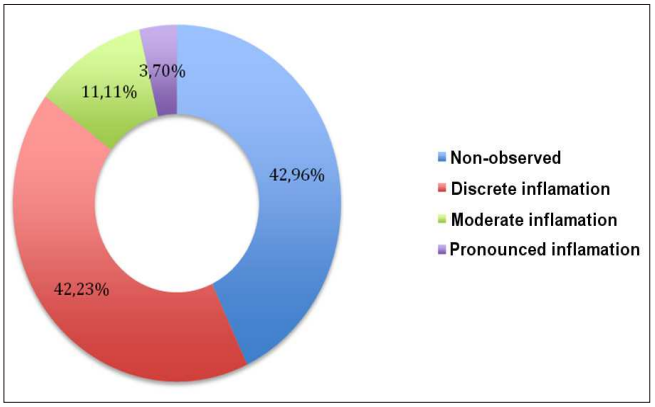

Figure 5 - *Acute inflammatory response -% neutrophils.*

literature, range from 1% to 10%[23,24]. The wire presents logistical problems with placement and positioning performed a few hours before surgery, causing inconvenience, anxiety to the patient, delay and sometimes suboptimal location. Since these are mobile structures in relation to breast parenchyma, they may be associated with important perioperative complications, emphasizing the movement of the needle / wire into the breast, especially in more fatty ones; there are even reports of their migration into the pleural cavity with occurrence of pneumothorax[25]. They can also be involuntarily transected or broken by the surgeon during the procedure, rendering the location during operation difficult or impossible and even generating the presence of iatrogenic "foreign bodies"[26].

The marking of impalpable breast lesions with radioisotopes (Radioguided Occult Lesion Localization - ROLL), has been used. However, it has the disadvantage of requiring a locating procedure in a different time of the surgical procedure, high cost, use of specialized radiopharmaceuticals and the need for a gamma radiation detector in the operating room[5]. It is also important to note that the use of iodinated contrast media reported in the original technique can lead to fat and skin necrosis, as well as skin ulcerations, compartment syndrome and allergies due to extravasation of contrast into the subcutaneous tissue[5,27]. The time between injection and surgery is too short, causing embarrassment to the treatment schedule.

Vital dyes such as toluidine blue [28], Blue Evans and Vital[18], methylene blue, green isocyanate[28] and India ink were used to identify subclinical lesions because of their low cost, but are ineffective because they diffuse easily in the breast parenchyma and should have its inoculation performed immediately before surgery, and the diffusion hinders proper identification of the segment to be removed. But coal, which has been used as a dye in a few places, does not offer the diffusion problem, being inert or with minimal diffusion.

Prehistoric people (the Otzi) have made use of tattoos associated with acupuncture for the purpose of marking of points and treatment of chronic diseases suffered by man of the ice age. At present, coal was introduced as a means of marking impalpable breast lesions around the year 1979, the Karolinska Hospital, Stockholm, Sweden. In 1983, Svane[19] published a work describing the stereotactic technique for preoperative marking on 53 patients with non-palpable lesions. The stability of staining with coal solution over time becomes very attractive. Being particulate and not soluble in water, it remains on the path and does not spread to adjacent tissue, in contrast with what occurs with other dyes. It is biologically inert, readily identifiable to the naked eye, and in most cases the coal is not identified radiographically.

In agreement with several previous studies, this research supports the use of coal as a efficient marker, of easy viewing by both surgeon and pathologist. Among the 135 specimens analyzed, we detected the lesion and coal at its periphery in 133 samples. On one sample without target lesion, the amount of coal was minimal, suggesting injection failure. With respect to the other sample without lesion, the amount of observed coal was abundant, and the specimen was exclusively represented by adipose tissue, suggesting coal location in an inappropriate site.

Few reports in the literature evaluated the impairment of difficulty in interpreting histopathological specimens labeled with coal suspension[1,4,11,20]. In the present study we did not detect any difficulty to the interpretation for the final pathological diagnosis. There was a case of intraductal carcinoma with displacement artifacts, which, at conventional optical microscopy, raised doubts as to the presence or absence of infiltrating lesion. An immunohistochemical assessment was necessary, confirming a diagnosis of intraductal lesion. These morphological findings are reported as coming from previous interventional procedures, needle biopsy or needle aspiration, not being directly related to the presence of carbon particles.

Mullen *et al.*[4] compared needle and coal marking in 2001. Surgeons reported large coal preference, since displacement of the needle occurred, as well as errors in the correct location of the lesion by using the needling technique[4].

There are few disadvantages of using carbon as a means of marking of non-palpable lesions reported to date. Among them there are scattered reports of cases with low grade "foreign body" reaction[20], without significant adverse reactions, even after months and years of follow-up[1,4,6,11]. According to reports in the literature, the appearance of granulomatous reactions can occur when the resection of the marking path is not accomplished in six months[4,12,13], time during which the coal is phagocytosed by macrophages, with little or no inflammation[29].

To date, there are no known studies that quantitative and qualitative analyze the inflammatory response to coal or distribution of it in relation to pathological lesions.

In this study we conducted quantitative and qualitative evaluations of the inflammatory response and we observed "foreign body" granulomas in all samples, regardless of the amount of coal, contradicting data obtained in other studies, which found minimal or absent reaction to "foreign body"[1,4,6,11-13,20].

Regarding lymphocytic inflammatory response, 5.19% of the samples showed no lymphocytic infiltration, in 82.22% it was mild, and moderate in 12.59%.

As to the acute inflammatory response, we observed absence of neutrophilic exudation in 42.96% of the specimens, discrete in 42.22%, moderate in 11.11% and severe, with microabscesses, in 3.7% of cases. Such an occurrence is unusual because the solutions used are theoretically sterile and should not cause any acute inflammatory reaction.

Another problem faced by pathologists during the histopathological examination is that during microtomy the coal can render tissues more resistant, and the lesion be distorted or hidden[4]. In this series we observed five lumps of coal, with paralesional position, which did not generate interference in the morphological analysis.

More recently, three cases of suspicious mammographic alterations have been reported, coinciding with previous coal marking sites, whose deeper investigation returned coal granulomas[29].

This study supports the usefulness and ease of the method as an effective marker of non-palpable lesions, with low cost, high effectiveness, subject to histological analysis, besides being easy to perform. It is comfortable for the patient and a great help to the surgeon and pathologist in locating these lesions. Therefore, this method should be most widespread and used in medicine.

In conclusion, coal marking was feasible and effective for the radiologist, the surgeon and the pathologist, had low cost, was comfortable to the patient and surgical team, of easily scheduling and took more time from the marking procedure and surgical removal; regarding impairment or not to the histopathologic diagnostic interpretation, the method was very effective, generating no diagnostic difficulties; quantification of inflammatory response to the presence of coal in most cases was chronic inflammatory reaction of varying degree, and in more than half, acute inflammatory reaction, including foci of abscesses; in relation to target lesions, the paralesional distribution of the coal was the most frequent and the infiltrating malignant lesions were those more likely to present intralesional distribution of coal.

R E S U M O

Objetivo: *Analisar a eficácia da suspensão de carvão inerte na marcação de lesões impalpáveis mamárias e as alterações morfológicas associadas ao seu uso, além de determinar se há ou não prejuízo na interpretação destas lesões pelo patologista.* ***Métodos:*** *Foram atendidos 135 casos de lesões impalpáveis mamárias, previamente marcadas com suspensão de carvão. As lâminas coradas pelo método de HE contendo pigmentos de carvão foram analisadas ao microscópio óptico, onde se realizou avaliação quantitativa e qualitativa da resposta inflamatória e determinação da presença ou não de prejuízo ao diagnóstico anatomopatológico. Os índices avaliados quantitativamente quanto à resposta inflamatória foram as contagens de linfócitos, células gigantes e neutrófilos, todos observados e quantificados em campos de grande aumento. Foi, ainda, avaliada a quantidade e distribuição de carvão presente nas lesões.* ***Resultados:*** *Na avaliação quantitativa e qualitativa da resposta inflamatória observou-se, independente da quantidade de carvão, granulomas a "corpo estranho" em todas as amostras. Em relação à resposta inflamatória linfocitária 5,19% das amostras não apresentaram infiltração linfocítica, sendo que em 82,22% ela era de discreta intensidade e em 12,59% de moderada intensidade. Quanto à resposta inflamatória aguda, observou-se ausência total de exsudação neutrofílica em 42,96% dos espécimes, exsudação neutrofílica discreta em 42,22%, moderada em 11,11% e acentuada, sob forma de microabscessos, em 3,7% dos casos. Nesta série foram observados cinco nódulos de carvão, posicionados paralesionalmente, e que não geraram interferência na análise morfológica.* ***Conclusão:*** *Este método é marcador eficaz de lesões impalpáveis, de baixo custo, alta efetividade, sem prejuízo à análise histológica, além de ser de fácil execução; é confortável para a paciente e de grande auxílio ao cirurgião e patologista na localização destas lesões.*

Descritores: *Carvão vegetal. Morfologia. Mama. Neoplasias. Neoplasias da mama.*

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