Review on Practical Approach in Multiple Breast Carcinomas: Does Each Focus Matter?

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Rezumat

Reevaluarea unei abordări practice în carcinoamele mamare multiple: cât de mult contează fiecare focar tumoral?

Carcinomul mamar reprezintă cea mai frecventă tumoră malignă ce se dezvoltă la sexul feminin. De obicei, este o tumoră unică dar, uneori, poate fi multiplă. Datele din literatură cu privire la incidența, definiția, profilul morfologic și molecular, tratamentul și prognosticul carcinoamelor mamare multiple sunt contradictorii, de aceea, acest articol prezintă o abordare practică pentru cei implicați în diagnosticul și tratamentul unei astfel de leziuni care, poate prezenta heterogeneitate morfologică și moleculară, acest lucru având un impact asupra managementului și prognosticului. De aceea, este esențial ca focarele tumorale multiple să se raporteze separat și să fie luate în considerare în momentul unei decizii terapeutice.

Cuvinte cheie: focare tumorale multiple, carcinom mamar

Abstract

Breast carcinoma is the most frequent form of cancer encountered in women worldwide. In the routine practice, most of breast carcinomas are diagnosed as unifocal, however, a variable proportion is represented by multiple tumor foci. Since data regarding the incidence, definition, morphological and molecular profile, treatment and

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prognosis of multiple breast carcinomas are currently contradictory we are presenting a practical approach for pathologists dealing with such a lesion, which may display morphological and molecular heterogeneity among multiple foci with an impact on management and prognosis. Multiple tumor foci should be reported separately and taken into account when establishing the treatment.

Key words: multiple tumor foci, breast carcinoma

Introduction

Breast carcinoma is the most frequent form of cancer encountered in women worldwide, as it represents 23% of all cancers globally, with 1.38 million newly diagnosed cases/year, and 28% of all forms of cancer in Europe (1). In Romania, breast cancer is a major public health issue as the incidence of breast cancer increased steadily during the last decades in Romania, from 25/100.000 women in 1988 to 61.1/100.000 in 2008 (2).

In the routine practice, most of breast carcinomas are diagnosed as unifocal, while a variable proportion is represented by multiple tumors. Data available in the literature regarding the incidence, definition, morphological and molecular profile, treatment and prognosis of multiple carcinomas are currently contradictory and insufficient for best patient management. We are presenting here a practical approach for pathologist dealing with breast cancer diagnosis and management, based on the review of international literature and several studies that we have performed and published on this topic aiming to demonstrate that each focus of multiple breast carcinoma matters.

Incidence

The incidence of multiple breast carcinomas varies in the English medical literature between 6.1 and 77%, due to differences in definition, inclusion/selection criteria, preoperative diagnostic methods (the incidence is 15% when detected with mammographic examination and 35% when detected with MRI and ultrasound) and, last but not least, differences in sampling methods and their correlation with preoperative radiologic examinations used in different onco-

logic hospitals (3,4). The incidence of multiple breast carcinoma detection has grown in the last decades with the advent and universal use of preoperative imaging diagnostic methods (mammography, ultrasound and MRI). However, even the use of these imaging methods does not guarantee the discovery of all tumor foci, as some of them are detected only following macroscopic and/or microscopic examination during breast specimen sampling. More recent studies that histopathologically analyzed consecutive cases using the "wide section" method have revealed the presence of multiple, secondary foci in most breast carcinoma patients (5).

Since the data regarding the incidence of multiple breast carcinomas in Romania were missing in English literature, we performed a retrospective study including 1787 consecutive cases diagnosed with breast carcinoma in the Pathology Department of Tîrgu Mures (2002-2012). We determined the global incidence of multiplicity in breast carcinomas and compared the incidence of multiple carcinomas in 2002-2006 (when the "traditional" breast specimen sampling method was used) with the incidence in 2007-2012 (after we implemented the new sampling method, also named the "multidisciplinary" method, which correlates radiologic and histological appearances) (6). The global incidence of multifocality in our Pathology Department was 22.55% (403/1787 cases). The incidence of multiple breast carcinomas increased significantly between 2007-2012 compared to 2002-2006 (31.04% versus 13.31%) ($p < 0.0001$), starting with the introduction of the concepts of radiologic appearance-correlated sampling and multidisciplinary team and it is similar to data reported in the international medical literature (6).

Definition and Terminology

Multiplicity (multifocality/multicentricity) in breast carcinomas is a controversial subject in the literature, as there is no international consensus on the definition. The first author who referred to the presence of multiple breast tumors was John Hunter, in 1839, in "Lectures on the Principles of Surgery", where he suggested the possibility that the multifocality of breast carcinoma originated in multiple foci within the same breast that were rarely detected simultaneously (7). Since then, various definitions have been applied to report these tumors, but the clinical significance by using different definitions is still unknown (3,8-17).

Traditionally, multiple carcinomas have been classified in two categories: multifocal (MF) and multicentric (MC). These definitions were not applied in a uniform manner and these two terms are sometimes used together, which can lead to confusion. Also, the distinction between MF and MC carcinomas was made using several criteria: topographic (MF: tumor foci located in the same quadrant /MC: in different quadrants) (3,17-19); histological pattern (similar: MF/different: MC) (20,21); tumor origin - Vlastos and Middleton defined MF tumors as multiple foci of the same tumor, whereas MC involved multiple foci of origin (11,22). A delimitation between MF and MC carcinomas was also attempted by using an arbitrary distance between tumor foci, for instance: <2 cm (MF) or >2 cm (MC) (9,23); >3 cm (MC) (10); <4 cm (MF) and >4 cm (MC) (3); <5 cm (MF) and >5 cm (MC) (8,17,18). In order to consider a tumor as MF, some authors recommend a minimum distance between foci: >1 mm (24), >4 mm (25,26), >5 mm (27,28). Some of these authors included both (in situ and invasive carcinomas) in their definitions, others only included invasive carcinomas. Most of these authors only took into account grossly visible foci and ignored microscopic foci. Other authors (12,13,14,29) used both terms together, without making a difference between MF and MC by avoiding "quantitative" delimitations. They considered breast carcinomas to be multiple

when multiple invasive foci separated by benign breast tissue are seen, regardless of the distance between foci; topographic criteria and distance between tumor foci are considered by these authors to be parameters of debatable biological significance (5). This definition suggests that according to more recent studies, the morphology and molecular profile of multiple tumor foci are more important parameters to determine the prognosis than is the location or the distance between multiple foci within the breast.

Origin

As far as their origin is concerned, the development of multiple foci in breast carcinoma could be explained by two mechanisms: these foci may develop as multiple independent tumors, in which case tumors could display different molecular and phenotypic features (polyclonal), or by intramammary dissemination of a single tumor, which would render them identical (monoclonal) (30). Establishing the origin of the multiple breast carcinomas, as well as carrying out clonality studies, are important due to their therapeutic perspectives. However, we have to keep in mind that sometimes multiple independent tumors may have similar morphological and molecular profile within different foci and on the other hand that multiple tumors with identical profile may change in time and differentiate into clones with different profiling especially when metastasizing.

Sampling Method and Pathology Report

There are two sampling methods which are generally used in diagnosing multiple breast carcinomas: 1. the randomized biopsy of each breast quadrant not involved by the tumor, along with sampling the principal tumor, which is histologically examined (15,18,31,32) and 2. the serial gross section method in which the entire breast tissue is examined, allowing a more precise determination of the relationships between lesions, with or without radiologic examination (5,8,9,21,33). The incidence of multiple tumors when using the serial

section method varies between 21% and 63%, with a mean value of 43%, while when using randomized quadrant biopsy, the incidence of multifocality varies between 11% and 41.6% (with a mean value of 26%) (25). On the other hand, MD Anderson Cancer Center (USA), uses a more complex method in detecting multiple breast carcinomas, consisting of a correlation between preoperative radiologic appearance and radiographic re-examination of the serial sections performed during sampling, with findings of the gross examination and a very detailed sampling of all suspected tumor (34). This method is, however, extremely costly, as it requires the presence of the radiologist, surgeon, and pathologist during surgery, highly trained personnel and sampling a high number of tissue fragments. In the Pathology Department of Tîrgu Mureș, we adopted the MD Anderson method in 2007. Moreover, a detailed mapping of each sampled focus is performed in each case in order to allow the pathologist to better define, localize, and correlate the presence of multiple tumor foci. (Fig. 1)

The latest editions of AJCC and TNM system define ipsilateral synchronous multiple breast carcinomas as the presence of at least two invasive tumor foci located within

the same breast, macroscopically distinct, and assessable using clinical and pathological methods (35,36). The multiple foci should only be assessed in terms of their number, which should be reported between parentheses in the final pathology report. Also, according to the above-mentioned international guidelines, these criteria did not apply to tumors with a single macroscopic focus associated with several separate microscopic foci, which are called "satellite tumors" (35,36). These guidelines recommend the use of the maximum diameter of the largest tumor focus in multiple carcinomas, rather than the sum of all diameters when reporting the tumor in the final pathological report. In a study published in 2016, we aimed to determine the optimal method for tumor size assessment in multiple breast carcinomas, correlated with the development of axillary lymph node metastases. (Fig. 2)

By using the aggregate diameter method, some of the patients enrolled in this study were upstaged to a higher T stage, but this method of assessing tumor diameter does not correlate with a higher rate of axillary metastases and should not therefore be used in the TNM staging of multiple breast carcinomas (37). The diameter of each tumor foci should

Figure 1. The "map" of the sampled sections filled in by the pathologist; the significance of the each sampled fragment and each microscopic section, following a preexistent code; e.g.: L2 (slide 2)= breast tumor adjacent to the tumor; L3, L4, L10= tumor; L5= superior resection margin; L6= Tumor + lateral resection margin; L9= Tumor+ medial resection margin; L8= inferior resection margin

Figure 2. The size of tumor foci in multiple breast carcinomas assessed by using two methods: (1) Largest diameter of the largest tumor focus (LD); (2) Aggregated diameter of all tumor foci appearing in one specimen (AD)= the sum of the maximum diameters of all individual tumor foci

be included in the pathology report, however, since it gives the oncologist an idea about the total volume of the tumor that should be treated; on the other hand, when staging, the largest diameter of the tumor foci should be used.

Morphological Profile

Multiple foci tumor can be, according to some studies, different from a microscopic standpoint (22,38,39) and polyclonal (40), but other studies state the contrary and uphold the monoclonal theory, according to which these foci are in most cases morphologically identical (22). (Table 1)

The morphological features (histological tumor type and grade) play an important role in deciding the best adjuvant treatment together with the molecular profile. Also, these morpho-

logical parameters are involved in assessing the prognosis and risk of relapses. The European Guideline for quality assurance in breast cancer screening and diagnosis (2006) only reports the features of the main tumor (41). According to the AJCC/UICC TNM system's latest edition, the College of American Pathologists (2009) and the ESMO guidelines, only the largest tumor focus should be selected for classification, grading, and staging (35,36,42,43). Clinical decisions in systemic adjuvant therapy for breast cancer are presently based on morphological criteria of the largest tumor focus, ignoring those of the smaller simultaneous cancers even though the heterogeneity of individual foci in multiple carcinomas has not been widely studied (44-46).

In a recently published study, we aimed to assess whether the morphological appearance (i.e. histological tumor type and histological grade) of simultaneous invasive breast carcinoma foci is different, since it is known that adjuvant therapy is established according to these parameters (47). We carried out a retrospective study comprising 498 consecutive patients admitted to our institution, diagnosed with breast carcinoma and surgically treated between 2007-2009 out of which 91 (21.77%) had multiple invasive tumor foci. In multiple tumors, each lesion was analyzed separately by two authors of this paper (SS and MB). In all cases defined as multiple tumors, we were able to detect an index, principal tumor with the largest diameter of all foci, and additional foci (which always had a smaller size). The histological tumor type of the additional foci was different from the index tumor in 12.08% of the cases. In 9 cases (9.89%) we encountered a

Table 1. Studies analyzing discordance in histological types and grades between multiple tumor foci in invasive multiple breast carcinomas

Authors	No. of cases analyzed	Proportion of cases with different histological types (%)	Proportion of cases with different microscopic grade (%)
Egan et al. (20)	118	25	Unknown
Dawson et al. (30)	24	37.5	Unknown
Andea et al. (38)	101	15.8	4.9
Middleton et al. (22)	32	3	0 (zero)
Moutafoff et al. (39)	234	9	Unknown
Choi et al. (28)	65	37 (histological pattern)	12

different histological grade compared to the index tumor. Multiple breast carcinomas may show pathological heterogeneity among foci. Tumors with histological types bearing a favorable prognosis and lower histological grades have been encountered in association with simultaneous foci with poorer prognosis and higher histological grade. By reporting only the features of the main tumor, the chances of the patient to receive adjuvant therapy could be diminished (47). By following the international recommendations (35,36,41), at least 8 cases from our study (with only the index tumor previously reported) would have been under-treated (47). We believe that reporting the morphology (histological type and grade) of each tumor focus is imperative.

Molecular Profile

International therapeutic guides recommend adjuvant endocrine therapy (Tamoxifen) in patients with breast carcinoma in which the tumor expresses estrogen receptors (ERs) and/or progesterone receptors (PRs), as well as anti-human epidermal growth factor receptor 2 (HER2) therapy (Trastuzumab) in HER2-positive cases (43, 44, 45, 48). An increased proliferative index (Ki-67), a high histological grade, and ER/PR negativity are factors that indicate the use of chemotherapy (43,46). The assessment of these markers is thus important,

because it provides data that are helpful in establishing the treatment and prognosis of breast carcinomas. TNM 2012, AJCC 2010 and CAP guidelines recommend that in multiple tumors, biological parameters (ER, PR, Ki-67, HER2) should only be assessed in the largest tumor focus, whereas additional tumor foci are only reported and assessed when they differ morphologically from the main tumor (35,36, 42). There are no current international standards regarding work protocols in these multiple carcinomas. However, not enough attention has been paid in the literature to the heterogeneity of multiple carcinoma foci. There are few studies that assessed histological and immunohistochemical features of tumor foci in multiple carcinomas, with contradictory results and variable conclusions (22, 28, 30, 49, 50,51). (Table 2)

We aimed at analyzing all tumor foci from an immunohistochemical standpoint and at highlighting concordances or mismatches between them on a series of 806 cases (out of which 155 were multiple tumors) consecutively diagnosed with breast carcinoma between 2007-2012 in Tîrgu Mureş. In our study, we found mismatches in 11.61% to 29.03% of cases, depending on the analyzed biological parameter (52). Of the 155 cases analyzed, 61 (39.35%) were morphologically and immunophenotypically concordant. Of the 23 cases (14.83%) in which the multiple foci were

Table 2. Studies summarizing and comparing the immunohistochemical (IHC) profile of multiple breast cancers

Study	No. of cases	Mismatches in immunohistochemical profile n (%)				Mismatches in histology (%)
		ER	PR	ki67	HER-2	
Middleton (22)*	14	0	0	2 (14.28%)	0	3%
Garimella (50)	18	0	2 (11%)	ND	ND	ND
Choi (28)	65	2 (3%)	7 (11%)	ND	4 (6%)	37%
Pekmezci (51)**	51	4 (7.8%) (ER and PR were assessed together)		11 (22.4%)	1 (2%)	25.49%
Buggi(49)***	113	5 (4.4%)	18 (15.9%)	17 (15%)	11 (9.7%)	No

ki67 Cut-off values used in the analyzed studies: * low <17%; moderate 17-35%; high >35%; ** low <10%; moderate 10-20%; high >20%; *** low <20%; high >20%

Abbreviations: ER, Estrogen Receptor; PR, Progesterone Receptor; ki67, Proliferative index; HER-2, Human Epidermal Growth Factor Receptor 2; ND= Not Determined; n, number of cases; %, percentage

morphologically different, in 10 cases (6.45%), the foci were immunohistochemically similar but were different in 13 (8.38%). In 71 cases (45.8%), although the multiple foci were morphologically identical, there were immunohistochemical mismatches. Our study thus underlines the importance of independently assessing and reporting each tumor focus in multiple carcinomas, morphological features not with standing.

Comparison between primary tumor and axillary lymph nodes regarding morphology and molecular profile

We and other authors have demonstrated that multiple breast carcinomas can differ among foci regarding histology and molecular profile (28,49,53). No studies however analyzing the impact of this heterogeneity on axillary metastases have been performed in multiple breast carcinomas. For this goal, we have recently performed a study on 806 consecutive breast carcinoma cases out of which 155 were multiple tumors. Out of the 155 multiple carcinomas, 115 cases displayed identical histological type and grade in all foci, while 40 cases showed morphological heterogeneity; of these, 11 showed mismatches only between the histological type of the multiple tumor foci, 16 showed mismatches only between histological grade and 13 cases presented with mismatches between both histological type and grade (54).

Out of the cases in which only grade mismatches appeared (but which had the same histological type) and which determined axillary lymph node metastases (14 out of 16 cases), the metastases had the same histology as the multiple breast tumors. The histological grade of the metastases was identical to that of the highest-grade tumor in all cases. In 35.72% (5 of 14 cases), the grade of the metastases was identical to the grade of a smaller tumor than the index tumor. As far as the cases in which only histological type mismatches appeared (but which had identical grades) and which had axillary lymph node metastases (8/11 cases), the histological type of the metastases was homogeneous in 4 cases (regardless of the number of

metastases), but in the other 4 cases the histological type was heterogeneous (54).

The histological features of axillary lymph node metastases in multiple breast carcinomas correspond to the histological type with unfavorable prognosis and/or highest histological grade, which is not necessarily of the largest tumor focus (54).

Regarding the molecular profile, previous data revealed the instability of the tumor cell proliferation index throughout the metastatic process, which could have clinical consequences and can result in therapeutic changes. Also, comparing the ER, PR, and HER2 status in the primary tumor and paired lymph node metastases, several studies found a variable rate of instability (55-58).

We also aimed to demonstrate that in breast carcinomas the tumor profile is not stable during the metastatic process, with impact on therapeutic decisions. In a recently published paper, we analyzed ER, PR, HER2 status and Ki67 index in 41 primary unifocal (PU) and 37 primary multiple (PM) breast carcinomas with identical immunohistochemical profile among multiple tumor foci and the matched axillary lymph node metastases (59). We excluded from this study cases of multiple breast carcinomas in which the tumor foci were molecular heterogeneous (because we expect these tumors to be associated with heterogeneous lymph node metastases from a molecular point of view). We defined concordant cases those in which the primary tumor (PU or PM) and lymph node metastases displayed identical positivity or negativity for ER, PR, HER2, Ki67 and discordant cases in which there was a mismatch in at least one biological parameter between PU and PM tumor and lymph node metastases. Also, we defined concordant cases those in which the molecular profile (based on the immunohistochemical evaluation of the ER, PR, HER 2, Ki67) was concordant between PU and PM tumor and lymph node metastases, and mismatch cases those in which the molecular profile of the primary tumor differed from the one of the lymph node metastases in at least one lymph node. The positivity for the biological markers is not stable during the metastatic

process, in this study the total rate of discordant cases was 92.7% in PU tumors and 75.7% in PM homogeneous tumors ($p=0.058$, $OR=0.245$, $95\%CI=0.06-0.991$). The total rate of shifted case was 64.9% in PM tumors and 82.9% in PU tumors. The highest rate of shifting was encountered from Luminal B-like to Luminal A-like. In 11 out of 37 (29.7%) PM, and in 17 out of 41 (41.5%) PU cases the subtype shifted to a poorer one regarding prognosis (59). In conclusion, the patients in whom the primary tumor is hormone receptors negative and/or HER2 negative but positive for these markers in the axillary lymph nodes could become eligible for hormonal treatment and/or trastuzumab treatment which may significantly improve the patient's outcome. This is the reason why performing the molecular profile is mandatory in both primary tumor and lymph node metastasis on Tru-cut biopsy specimens at the moment of diagnosis and before establishing the management.

Treatment

A multidisciplinary team that includes at least a radiologist, an oncological surgeon, a radiotherapist, a medical oncologist, and a pathologist establishes the therapeutic strategy and the sequence of therapeutic stages in multiple breast carcinomas. When confronted with the presence of a multiple breast carcinoma, clinicians encounter several difficulties concerning surgical management, which involves local/ regional treatment leading to a wide excision of breast tissue. There is no standard method in the surgical management and treatment of multiple carcinoma. However, lately, a more conservative approach has been adopted consisting of subtotal mastectomy/ lumpectomy /wide sectorectomy and management of the axilla (sentinel lymph node biopsy in order to prevent total axillary lymph node dissection when the sentinel lymph node is negative)(60). Also, since these cases are associated to a higher extent with axillary lymph node metastases (in which case chemotherapy is recommended), they would benefit more from adjuvant chemotherapy than patients with unifocal tumors.

Prognosis

Multifocality is not listed among the "traditional" prognostic factors (tumor size, histological grade, axillary lymph node status) or among the second generation ones (ER, PR, Ki67 index and HER2 status) in breast carcinoma (5), even if in 2014, a systematic review and a meta-analysis which focused on the effect of multifocality and multicentricity on outcome in early stage breast cancer and in which 22 studies were evaluated (with a total of 67,500 women included) revealed that in univariable analyses, multifocality was associated with worse disease specific survival and worse local rate recurrence at 10 years. Also, in multivariable analyses, multifocality was associated with significantly worse overall survival (61). However, studies regarding the prognostic significance of multiple foci in breast carcinomas are contradictory and the differences in data could be caused by many factors, such as: case inclusion criteria, variations in the definitions used, different statistical analysis methods and different interpretations (4,11, 13, 20, 22, 26, 27, 62- 68). (Table 3)

We aimed to analyze survival in multiple breast carcinomas compared with unifocal ones, in order to assess the impact of multifocality on prognosis. We conducted a retrospective study that included 460 consecutive cases of breast carcinomas admitted to the Pathology Department Tîrgu Mureş between 2002-2006. Out of these, 69 (15%) cases were multiple carcinomas, and 391 (85%) cases were unifocal carcinomas. In our study, multifocality was associated with a lower general survival at 5 years and at 10 years, yielding a higher mortality rate and a lower median survival rate, but did not constitute an independent prognostic factor in multivariate analysis (69).

Conclusions

Firstly, we have demonstrated that the incidence of multiple breast carcinomas in Romania is similar to the one reported internationally if the radiological-pathological sampling method is applied and a good correlation among data and the members of the multidisciplinary

Table 3. Studies regarding the prognostic significance of multiple foci in breast carcinoma (MFMC carcinoma vs. UF carcinoma)

Authors	Survival indicators
Worse prognosis	
Egan, 1982 (20)	Mortality ↑
Dabakuyo, 2008 (63)	Mortality ↑ 5 and 10-year survival ↓
Yerushalmi, 2009 (4)	Survival rate slightly ↓
Boyages, 2010 (62)	Mortality rate ↑ 5 and 10-year survival ↓
Weissenbacher, 2010 (68)	Mean survival ↑ ↑ Local recurrence rate ↑ Rate of distant metastases
Tot, 2011 (67)	↑ Risk of death due to breast carcinoma
Ustaalioglu, 2012 (24)	↓ Disease free survival
Pekar, 2013 (53)	↓ Breast Cancer Specific Survival
No influence on prognosis (no statistically significant differences between compared groups)	
Vlastos, 2000 (11)	Breast Cancer Specific Survival at 10 years Disease-Free Survival
Middleton, 2002 (22)	Disease-Free Survival at 5 and 10 years
Oh, 2006 (65)	Overall Survival at 5 years
Litton, 2007 (64)	Overall Survival, Relapse-Free Survival
Joergensen, 2008 (13)	Overall Survival
Cabioglu, 2009 (27)	Disease-Free Survival at 5 years
Rezo, 2011 (26)	Overall Survival, Progression Free Survival
Lynch, 2012 (66)	Relapse-Free Survival, Breast Cancer Specific Survival – worse prognosis at 5 years (only in UVA, not in MVA – other factors are associated with ↓ survival rates)

team is met in every case.

Secondly, we have demonstrated that there is a morphological and molecular heterogeneity among multiple tumor foci and this should have an impact on management. So, each focus of the multiple breast carcinoma matters and we strongly recommend that each focus should be investigated and reported, and therapeutical decisions should only be made by taking into the consideration tumor foci heterogeneity, even if the decision regarding the treatment in the presence of multiple tumors is still internationally debated.

Thirdly, we have demonstrated that both multiple and unifocal breast carcinomas are not stable during the metastatic process and even homogeneous multiple foci can change morphology and molecular profile when reaching the axilla, so we recommend markers to be evaluated and the molecular profile to be done on the axillary lymph node metastases also.

Finally, although multicentricity does not constitute an independent prognostic factor in multivariate analysis, multiple breast carcinomas have a worse prognosis than unifocal ones and this should be taken into consideration when establishing the treatment.

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