

Routine assessment of prognostic factors in breast cancer using a multicore tissue microarray procedure

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Abstract We propose multicore tissue microarray (TMA) as an alternative to whole section for routine assessment of prognostic factors in breast cancer. Since 2004, we introduced the multicore TMA for testing estrogen (ER) and progesterone receptors (PR), proliferation activity by Ki67, and HER2 overexpression and amplification in routine work. At least four tumor foci were selected on the whole section, and a dedicated technician used a stereomicroscope for accurate sampling of the selected areas. To identify a specific case in the TMA, a separate file and a computerized reporting form with the TMA map were created. A preliminary pilot study comparing the TMA results with those obtained on whole sections showed the specificity of the procedure. Moreover, in everyday diagnosis, hormone receptors were repeated on full section when negative in TMA, without significant discrepancy. Retrospective analysis of the 237 breast carcinomas studied by TMA showed the expected correspondence of tumor-grade differentiation with the hormone receptor pattern, the proliferation activity, and HER2 immunohistochemical and FISH values. In conclusion, multicore TMA may be an efficient approach in the routine study of prognostic factors in breast cancer, significantly reducing costs, time, and burden of slides necessary to accomplish these mandatory tests.

Keywords Multicore TMA · Diagnosis · Prognostic factors

Introduction

The tissue microarray (TMA) is a technical procedure that allows combining tens to hundreds of paraffin-embedded tissue specimens into a single paraffin block, so that it is possible to have a great number of different tissues or of different pathological entities of the same organ onto a single slide for analysis at one time.

First described by Kononen et al. [10], the use of TMA has increased over the last few years as more researchers have recognized novel applications across a wide variety of scientific disciplines. In fact, TMA represents a solution for studying correlation of gene and protein expression in a large-scale tissues of either the same or of different origin [1, 5, 14, 16, 18]. It is commonly used in the development of diagnostic and prognostic indicators for clinical applications, with a wide range of techniques such as RNA in situ hybridization, fluorescence in situ hybridization, and immunohistochemistry [3, 10, 13, 20].

In breast cancer, the study of prognostic and predictive biomarkers has become a mandatory step of the pathological workup, so that for each case of breast carcinoma, pathologists are due to perform at least four immunohistochemical reactions to study estrogen (ER) and progesterone receptors (PR), HER2 protein, and at least a marker of proliferation activity such as Ki67. This involves at the same time a critical loss of precious material and a great burden of slides to be stocked in the pathology files [3]. In addition, in laboratories managing high number of breast cancer surgical specimens, the cost for this immunophenotyping in terms of technician's workload, costs of reagents, and time for evaluation of the reactions is very high.

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Table 1 Clinical data of 237 patients

Age >50 years	197
Age <50 years	40
pT1c	140
pT2	69
pT3	8
pT4	7
Local recurrence	7
Distant metastases	6
pNx	13
pN0	104
pN0(i+) SN	4
pN1mi	7
pN1	56
pN2	31
pN3	9
Infiltrating ductal carcinoma	156
Infiltrating lobular carcinoma	30
Infiltrating mixed histotype	19
Infiltrating special type	32
G1	63
G2	92
G3	82

Zhang et al. [19] conducted a feasibility study by investigating the efficiency and effectiveness of immunohistochemical and FISH analyses in TMA of breast cancers constructed using a single 0.6-mm core biopsy per specimen. They found a high concordance comparing the results obtained from both TMA and full section (95%) and asserted the reliability of an immunophenotyping evaluation based on the analysis of a single tissue core. Recently, Bhargava et al. [2] using four cores demonstrated that the FISH scores were consistent among the two to four cores in the majority of the cases and that the TMA and full-section results were concordant in 99% of cases.

Taking all these data together, we decided to introduce TMA technology in routine diagnosis of prognostic and predictive factors of breast tumors. In a pilot study, we confirmed the results reported in literature [2] on the reliability of four cores to reproduce the immunophenotyping and the *HER2* gene status of the tumor. As a result, we extended this approach to the everyday diagnosis of breast tumors. We in this study report the protocol used and the analysis in terms of costs, time, archival slide load and workload of our laboratory using multicore TMA technology in breast cancer immunophenotyping and FISH testing, comparing them to the traditional examination on full sections.

Materials and methods

Case series

Thirty-six TMAs were prepared for immunohistochemical and FISH tests from a total of 237 consecutive cases of carcinomas of the breast, comprehensive of 126 cases operated in the Breast Unit of the San Giovanni Battista-Molinette Hospital of Torino from December 2004 to October 2005 and of 111 outsource cases sent for immunohistochemical testing of prognostic factors from the Vito Fazzi Hospital of Lecce. Tumors with a diameter less than 1 cm were excluded from TMA preparation. Patients' clinical data are summarized in Table 1.

Selection of areas for punching cores

For each case, we selected the tumor foci for the TMA construction during routine diagnosis by marking them on the more representative hematoxylin–eosin (H&E)-stained slide with a waterproof pencil (Fig. 1). The number of selected



Fig. 1 Selection of areas for punching cores: the more representative foci are marked both at the edge and at the center of the tumor on the H&E-stained full section. Multicore TMA block: within the circles are

ER+/PR+/HER2– (1) and ER–/PR–/HER2+ (2) controls. The correspondent sections placed in the center of the slides show a homogeneous immunostaining

fields varied from four to seven per slide, depending on the heterogeneity both of the histological pattern and of the grade of differentiation of the invasive breast cancer component. Moreover, care was taken to select areas from both the leading edge and the center of the tumor. In some cases, two different slides of the same tumor (corresponding to two different tissue blocks) were marked. One area of normal tissue was selected whenever possible to build up an internal control.

TMA construction

A serial number followed by the year, for example TMA 1/2005, was given to each TMA so that it was possible to record it on the computer database used for routine diagnosis (Win Sap v.6.2.1 Engineering Sanita' Enti Locali Italia). The number of invasive breast carcinomas for each TMA varied from four to eight, depending on the weekly workload. When more than five fields of a tumor were selected, two lines of the same case were placed in the same TMA. TMA blocks were prepared and sliced by a dedicated technician and processed for immunohistochemical staining every Wednesday. Using the advanced tissue arrayer (mod. ATA-100, Chemicon International, Tamecula, CA, USA), tissue cylinders with a diameter of 1 mm were punched under the stereomicroscope from the specific areas of the "donor" block and brought into the "recipient" paraffin block. One core of an ER/PR-positive and HER2-negative (score 0) breast cancer and one core of an ER/PR-negative and HER2-positive (score 3+) amplified breast tumor were inserted as controls on the top of each recipient block (Fig. 1). The block was incubated in an oven at 45°C for 20' to allow complete embedding of the grafted tissue cylinders in the paraffin of the recipient block, and then stored at 4°C until microtome sectioning. For immunohistochemical reaction and for FISH, sections were collected on Super Frost Plus slides (Menzel Glaser, Braunschweig, Germany).

Immunohistochemical and FISH procedures

TMA slides were stained using the Ventana automated immunostainer (BenchMark AutoStainer, Ventana Medical Systems, Tucson, AZ, USA). The technician took care to collect the sections on the center of the glass to avoid nonhomogeneous staining of the cores. Six sections were cut: section 1 was stained with H&E; sections 2–5 were processed for immunohistochemistry to test the following prognostic markers: ER (monoclonal antibody, clone 6F11, prediluted, Ventana-Diaphat, Tucson, AZ, USA), PR (monoclonal antibody, clone 1A6, prediluted, Ventana-Diaphat, Tucson, AZ, USA), Ki67 (monoclonal antibody, clone Mib1, diluted 1:200, DAKO, Glostrup, Denmark), and HER2 (polyclonal antibody, A0485 c-erbB-2 oncoprotein, diluted 1:800, DAKO, Glostrup, Denmark).

Section 6 was processed for *HER2* gene FISH analysis using PathVysion *HER2/neu* probe kit (Vysis, Downers Grove, IL, USA). Briefly, sections were baked overnight at 56°C, dewaxed in xylene, dehydrated in 100% ethanol, and air-dried. Slides were then treated with proteases for 45–60 min, denatured, and hybridized overnight at 37°C with the probes (*HER2/neu/CEP17* SG probe 35-171060, Vysis, Downers Grove, IL, USA). Slides were washed with posthybridization buffer at 72°C, counterstained with 40,60-diamidino-2-phenylindole (DAPI), mounted, and stored in the dark before signal enumeration. For FISH analysis, the slides were examined by Olympus Bx41 fluorescence microscope equipped with a 100× oil immersion objective and a triple band pass filter for simultaneous detection of Spectrum Orange, Spectrum Green, and DAPI signals. Slides were first scanned at low power with a DAPI filter to recognize the TMA map. Areas of optimal tissue digestion and no overlapping nuclei were then selected in each core for counting. Forty to 60 cells for each case were counted. Cases were scored as amplified when the final ratio obtained in *HER2*/chromosome 17 signals was ≥ 2.0 .

Microscopic examination

As a pilot study, the first 20 cases were immunostained on conventional full sections as well. The same protocol was then used in the routine work when a case in the TMA was found to be negative for the expression of ER/PR.

The correspondence between the fields selected on the original slide and the tissue areas pulled out of the donor block was verified on H&E-stained slides (section 1). For study purposes, the immunohistochemical results of each single core of a tumor were recorded. The results were assessed following the method published by Barghava et al. [2] that used a four-core/case TMA similar to our procedure. Briefly, for ER and PR, both the mean percentage value of stained nuclei in the four cores and the most intensively stained score (expressed by a score, from 0 to 3) were evaluated and reported together with the "quick score" [12]. This is obtained by a combination of the proportion of cell staining plus the measure of intensity, and the final value predicts the response of tumors to endocrine therapy. *HER2* staining was assessed on a 0- to 3-point score [6] on each core. The highest score from different cores of the same tumor was reported. To control the results of *HER2* status evaluated by TMA, we correlated the immunohistochemical and FISH results to the hormone receptor status and the Ki67 values and to the histological grades (G1, G2, and G3) of tumors, which was performed using the Elston and Ellis grading system of diagnosis [7]. After the results were obtained from the pilot study for Ki67, we reported the value of the core with the highest proliferative activity. The cut-off value to identify high or low proliferating

Table 2 Correlation of immunohistochemical and FISH results on TMA and full section (preliminary pilot study of 20 breast carcinomas)

	ER		PR		Ki67		HER2		FISH	
	q.s. ≥ 3	q.s. < 3	q.s. ≥ 3	q.s. < 3	$> 20\%$	$< 20\%$	Score 2+/3+	Score 0, 1+	A	NA
TMAvs full section	17/17	3/3	16/19 ^a	4/1	12/13	8/7	1/2	19/18	1/1	19/19

q.s. Quick-score value 3 indicates a small (20%) chance of response to endocrine therapy (12); ER agreement 100%, PR agreement 85%

^aIn three cases, the final “quick score” was downgraded by 1 point due to either the lower percentage or the lower intensity of staining in the TMA compared to the full-section staining

activity was 20%, which corresponded to the mean proliferation of a series of 200 consecutive breast carcinomas.

Results

TMA's slides constructed with a limit of eight lines containing four to five tissue cores each (Fig. 1) were optimal in terms of staining consistency using the automated immunostainer, without discrepancy between central-placed cores and cores located at the periphery of the section. The presence of positive and negative controls and of normal tissues, when available, ensured the efficiency of the staining (Fig. 1).

Pilot study

The pilot study conducted on the first 20 cases showed high agreement between the results obtained on the full section and those obtained on TMA (Table 2). Perfect agreement was reached for ER, considering both the “quick score” and the percentage of stained nuclei, because in positive cases, the intensity of staining was almost homogeneous in all cores, the percentage of the receptor expression varied less than 5%, and no false negative results were obtained. Good agreement (85%) was achieved for PR. In fact, no false negative cases were observed in TMA. However, in two cases (10%), the percentages of receptor expression were 15 and 30% lower, respectively, in TMA than in the full section, and in three cases, the intensity varied by one point; thus, the final “quick score” value was downgraded in three cases of TMA. Good agreement (95%) was reached for Ki67 percentage results taking $\geq 20\%$ of positive nuclei as the threshold value to define high proliferating tumors. In fact, only in one case of infiltrating lobular carcinoma the percentage of proliferating cells counted on TMA was 6%, while on the full section was 20%. In one case (5%), HER2 was negative (score 1+) in TMA and moderately positive in the full section (score 2+), but FISH testing on the full section confirmed that the case was not amplified.

FISH results reached an agreement of 100%, considering cases as amplified or not amplified.

Routine multicore TMA

For routine tests, each TMA was constructed with four to eight cases, depending on the weekly workload (Table 3). The evaluation of TMA performance in terms of correspondence between the fields selected on the original slide and the tissue areas pulled out of the donor block showed that in about 90% of cases, the areas were correctly selected by the technician during TMA construction. As shown in Table 4, only one single core in 9.7% of cases and two cores in 0.8% of cases were not appropriate, having normal gland or fibrous tissue pulled out instead of the tumor tissue. In six cases, three or four cores floated off during the immunohistochemical processing (Table 4). The tumor in these six cases was highly sclerotic, and the cores were cracked on H&E-stained sections as well; thus, testing was performed on the full section. The results in three cases with only one core available on TMA slide corresponded to that on the full section. In cases where only one or two cores were lost, the reactions were not repeated on full section because the immunophenotype on TMA corresponded to that expected by the histology and the grade of tumors.

In the routine examination by TMA, we performed the ER and PR study on the full section of 22 cases completely negative on TMA. All these hormone receptor negative cases were G2 and G3, had a proliferation index evaluated by Ki67 ranging from 24 to 70%, and HER2 was overexpressed in nine cases. In seven of these cases, *HER2* gene was amplified. Only two out of 22 of ER/PR-negative cases resulted positive on the full section with 3% of ER and 2% of PR expression and an intensity score of 1 for both. In a case of infiltrating ductal carcinoma, only one core out of four was PR-positive. The reaction performed on the whole section confirmed that the tumor had two cell populations, one PR-positive at the leading edge, and one PR-negative in the center of tumor (Fig. 2a–d). Actually, a slightly

Table 3 Routine testing of prognostic factors in 237 cases of breast cancer: number of cases per single TMA, number of TMA and of full-section slides repeated for technical reason and/or for verifying ER/PR-negative immunostaining

Cases per TMA	4	5	6	7	8	Total number of TMA	Total number of slides
Number of TMA	2	6	7	11	10	36	
TMA slides	12	30	42	66	60		210
Repeated full-section slides	5	0	25	20	24		74

different cytohistological pattern was evident also at the retrospective examination on H&E-stained slide (Fig. 2e,f, insets). In other two cases, the tumor had a mixed ductal and lobular histotype, and the TMA was constructed with two lines of the same tumors. The immunohistochemical results were similar in the two histotypes (ER- and PR-positive, Ki67 <20%, HER2: negative).

All the 237 breast tumors were analyzed for HER2 overexpression and gene amplification by FISH (Table 5). Sixty-one cases were positive in immunohistochemistry (score 2+, 3+), and 33 of these (54%) exhibited *HER2* amplification. One case negative for HER2 overexpression (score 1+) was amplified with monosomy of chromosome 17 at FISH analysis. In addition, as reported in Table 5, HER2 was never overexpressed with score 3+ or amplified in G1 tumors, while 84% of HER2 with score 3+ were G3. HER2-negative cases showed high level of ER and PR in about 90% of cases against 56% of ER and PR nuclear expression in HER2 scored as 3+. Finally, 40% of HER2-negative cases showed high level of proliferation ($\geq 20\%$) against 84% of HER2 (with score 3+)-positive cases.

Diagnostic reports

To speed up the turnaround time of the diagnostic report as smoothly as possible, a computerized report for each TMA was created. The report included a map of the TMA slide examined with the line position of the cases and the number of cores for each lines, and a table summarizing the results, as shown in Fig. 3. A final separate report was then prepared for each single case of invasive breast cancer examined on TMA. On the top of this report, the following standard phrase was added: “Prognostic factor performed

on TMA number... Cores performed on block number... after selection of fields on H&E-stained sections of the tumor. The correspondence between the histology of selected fields and that of cores was assessed.”

Working time and cost analysis

The time and the cost for technical procedures and assessment of results using TMA or whole-section traditional procedure are reported in Table 6. The technical time required for preparing and sectioning one TMA constructed for example with eight cases is almost comparable to that needed for preparing eight traditional blocks and 40 slides. On the other hand, owing to the slide loads, two rounds of automated immunohistochemical staining are needed for testing eight cases using whole sections (32 slides) instead of one round using multicore TMA (four slides). The same is true for FISH because no more than six slides are processed every time by the technician to guarantee correct digestion and incubation. In addition, the time required for pathologists to read the immunohistochemical and FISH reactions is reduced by about 1 h. Finally, the costs are reduced by a factor of seven for immunohistochemical staining and FISH.

Discussions

The amount of work and responsibility for breast pathologists have notably increased in the last decade, since the assessment of prognostic and predictive factors in breast cancer has become mandatory for the therapeutic decision making. In the present study, we propose TMA of breast cancers constructed with multiple tissue cores of each tumor as an alternative to the whole section for studying ER, PR, proliferation activity by Ki67, and HER2 gene expression and amplification in routine work. In addition, we show that management of routine prognostic factors by multicore TMA carries on a significant reduction in reagent cost, technical time, and number of slides. For example, in our series of 237 breast carcinomas, we would have a burden of 1,185 slides to be stocked in the pathology file against 284 produced with 36 TMA.

Table 4 Technical performance of TMA on 237 breast carcinomas

Number of cores	Not appropriate	Missing
1	23 (9.7%)	15 (6.3%)
2	2 (0.8%)	3 (1.2%)
3	0 (0%)	3 (1.2%)
4	0 (0%)	3 (1.2%)

Number of cores either not appropriate or missing on slides

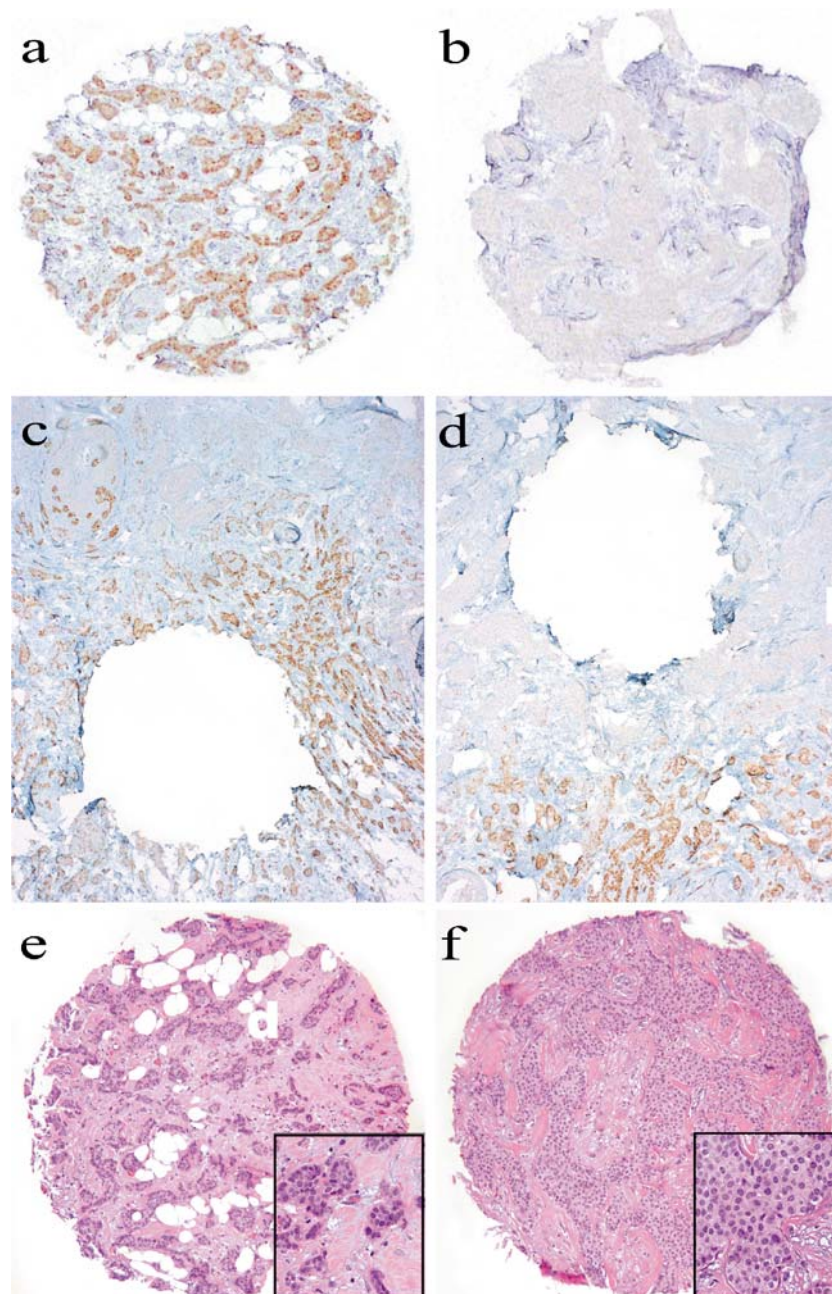


Fig. 2 Case of infiltrating ductal carcinoma with only one positive core out of four. The PR-positive core (**a**) comes from the edge of the tumor (**c**). PR-negative core (**b**) corresponds to a central area of the

tumor (**d**). Slightly different histological pattern is evident in H&E staining (**e**, **f**, and insets)

However, to reach a good performance of the TMA technique in the routine assessment of prognostic factors of breast cancer, the following points are, in our opinion, mandatory: (a) specific selection of at least four tumor foci for each case and evaluation of the correspondence of the selected foci on TMA and on the full section, (b) dedicated technician, (c) use of stereomicroscope for accurate sampling and extraction of the selected area from the donor block, (d) repetition on full section of cases with

negative hormonal receptor immunostaining, and (e) quality control of the results by comparing the immunophenotyping obtained by TMA with that expected by histology and grade of the tumor. In addition, to speed up the diagnostic report and to easily identify a specific case in the TMA, it was useful to create a separate file for TMA block and slide storing and a computerized reporting form with the TMA map. All these points, together with the sinking cost of the tissue microarrayer, obviously limit the

Table 5 Correlation of immunohistochemical expression of HER2 with HER2 gene amplification, tumor grade differentiation, hormone receptor status, and proliferation activity in the series of 237 breast carcinomas

HER2 score value (number of cases)	FISH amplified	G1	G2	G3	ER quick score ≥3	PR quick score ≥3	Ki67 >20%
Score 0 (79) (33%)	0 (0%)	24 (30%)	36 (46%)	19 (24%)	70 (90%)	68 (86%)	31 (39%)
Score 1+ (97) (41%)	1 (1%)	32 (33%)	42 (43%)	23 (24%)	90 (92.7%)	86 (88.6%)	41 (42%)
Score 2+ (36) (15%)	10 (27%)	7 (19%)	10 (28%)	19 (53%)	28 (77.7%)	30 (83.3%)	20 (55%)
Score 3+ (25) (11%)	23 (92%)	0 (0%)	4 (16%)	21 (84%)	14 (56%)	14 (56%)	21 (84%)
Total 237 (100%)	34 (14%)	63 (26%)	92 (39%)	82 (35%)	198 (84%)	154 (65%)	113 (48%)

use of TMA for routine to centers with high number of cases.

A limit to the use of TMA for routine immunohistochemical procedures may be the intratumoral heterogeneity and the different protein expression on cells from the leading edge vs those from the center of the tumor mass [4]. However, it has been shown that two to three cores of breast tumor are sufficiently representative of the entire lesion for assessing prognostic factors by TMA [4]. Moreover, even in highly heterogeneous tumors, such as ovarian cancer, the analysis of a single readable core

matches the staining pattern of a whole section more than 90% of the time and analysis of two core increases that value to more than 95% [15]. In the present work, the selection of at least four areas on the original H&E-stained slides, which was carried out during assessment of the histological grade, guarantees that the most significant intratumoral variations in terms of tubular formation, nuclear atypia, and mitosis number are identified. In addition, in agreement with others [2], in the pilot study we confirmed that four tissue cores highly represent the original tumor, even considering the possibility of tissue

TISSUE ARRAY N. TMA 8/2005

MAP:

CONTROLS

ER/PR: +

HER2: +

	1	2	3	4	5	6	7	8
a	•	•	•	•	•	•	•	•
b	•	•	•	•	•	•	•	•
c	•	•	•	•	•	•	•	•
d	•	•	•	•	•	•	•	•
e								
f								

RESULTS

LINE	HISTOLOGICAL NUMBER	PROGNOSTIC FACTORS NUMBER	ER%	PR%	HER2	KI67%
1	4552/05 1L1	5630/05	95 3+	5 1+	0	5
2	4762/05 2N1	5634/05	95 3+	75 2+	1+	14
3	4896/05 1N1	5635/05	90 2+	8 2+	0	29
4	5013/05 3N2	5640/05	90 3+	50 2+	0	10
5	5185/05 2N	5641/05	*0	*0	3+	40
6	5220/05 1N1	5643/05	95 3+	80 3+	0	7
7	5300/05 1N2	5644/05	85 2+	35 1+	0	24
8	5312/05 2N1	5645/05	95 2+	50 2+	1+	5

*Revised on the whole section.

Turin,

Signature:

Fig. 3 Multicore TMA computerized form reporting the serial number of TMA and the year of construction. The map of the TMA slide includes: the controls, the line position, and the cores performed for each case. Results are reported in the table. To each line/case

corresponds the histological and the prognostic factor number. The immunohistochemical results for each case are tabulated. For ER- and PR-negative cases (line 5), it is indicated that the reaction is repeated on the whole section (*)

Table 6 Comparative analysis of time and cost for studying eight cases using the traditional procedure on whole section and one TMA block (four immunohistochemical reactions, one FISH)

	Traditional procedure (eight blocks/40 slides)		TMA procedure (one block/five slides)	
	Time (h)	List price (€)	Time (h)	List price (€)
Block preparation	0.10	–	1.30	–
Sectioning	0.40	–	0.05	–
Automated immunoreactions and slides mounting	8	640	4	80
FISH reactions	96	5,600	48	700
Reading of immunohistochemical slides	1		0.30	
FISH slides	1.30		1	
Total	107.2	6,240	55.05	780

cores floating off on the slide during the immunostaining procedure. In fact, in only 2.4% of cases the reactions were repeated on the full sections because of missing cores on TMA, with a minimal impact on the final workload. Another possible bias to the use of TMA technology in routine is that a proportion of individual tissue cores may not be representative of the diagnostic section [8]. Our results show that when the TMA is prepared by a dedicated technician using a stereomicroscope for accurate sampling and extraction of the selected area from the donor block, the numbers of not appropriate cores are very low (1 core in 9.8% and 2 cores in 0.8% of cases).

Another important point is to ensure the reliability of the immunohistochemical results on multicore TMA. For this reason, first, we inserted control cores both for hormone receptors and HER2 analysis. Second, keeping in mind that the evaluation of hormone receptors is the parameter that highly influences prognosis and therapy of breast cancers [9], we decided to repeat the ER/PR test on whole section in case of negative results on TMA. A cut-off value for reporting hormone receptors as positive or negative has not been specifically defined in the literature; therefore, we use to report the percentage, the intensity, and the “quick score” [12] value that gives indication of the responsiveness of tumor to endocrine therapy separately. Using TMA, we showed some variations of PR “quick score” results compared to the full sections. The ER “quick score” was always confirmed. In addition, the retrospective analysis on the whole section of ER-negative tumors showed only in some cases a low percentage ($\leq 3\%$) of ER expression that would not modify the treatment decisions. HER2 immunohistochemical reaction showed instead more variable results (95%). On the other hand, FISH test on multicore TMA had a correlation to whole-section test of 100% as was shown also in a previous work [2]. For this reason and to guarantee a quality control on the HER2 immunohistochemical results, we decided to study by FISH every TMA, with a final cost that was affordable also for routine testing. In a previous work, we showed that G1 ductal carcinomas and

carcinomas of lobular and of special histological type did not show HER2 amplification even in the presence of protein overexpression [17]. The present results confirm these data with the exception of a case of lobular pleomorphic carcinoma that showed low level of gene amplification. Moreover, the retrospective analysis of the whole series of cases studied by TMA showed the expected correspondence of HER2 immunohistochemical values and FISH with the grade of tumor differentiation, the hormone receptor pattern, and the proliferation activity. In fact, the results of our series of breast cancers studied by multicore TMA match those recently published by Lal et al. [11] in a series of 3,655 breast carcinomas studied on whole histological sections.

In conclusion, multicore TMA technique may be an efficient and useful approach for routine management of prognostic factor in breast cancer, particularly for laboratories running high number of breast cancer surgical specimens, where the costs for this immunophenotyping in terms of reagents, technician’s workload, and time for evaluation of the reactions are quite high.

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