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Change in FGF-2 circulating levels after arterial embolization in patients with bone metastases

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Arterial embolization, aimed at the mechanical occlusion of tumor-feeding vessels, represents a satisfactory palliative therapy for bone metastases. In this study, we evaluated if the circulating levels of three factors related to the metastatic process change in response to embolization. Seven patients who underwent embolization of a single skeletal metastasis from carcinomas were analyzed prospectively. Circulating levels of Vascular Endothelial Growth Factor A (VEGF-A), Fibroblast Growth Factor 2 (FGF-2), and Tartrate-Resistant Acid Phosphatase-5b Isoform (TRACP5b) were evaluated before and after embolization at 1, 3, and 6 months. According to morphological and clinical evaluations, all the embolizations were successful. VEGF-A and TRACP5b did not show significant changes after the treatment. On the contrary, FGF-2 significantly decreased 1 month after the treatment. FGF-2 appears as a promising candidate for monitoring the efficacy of embolization in patients with osteolytic metastases.

Key words: bone metastases, transcatheter arterial embolization, angiogenesis, FGF-2

Metastatic carcinoma is the most common cause of destructive bone lesions in adults. Although breast carcinoma most frequently metastasizes to the bone, also prostate, renal, thyroid, and lung cancer represent favorable soil for the growth of skeletal metastases [1]. Skeletal morbidity includes pain, pathological fractures, and spinal cord compression, and requires palliative treatment [2]. Since osteolytic metastases are highly vascular, selective arterial embolization is considered a valid treatment option. The main purpose of this technique is to occlude as much of the vascular supply of a tumor with consequent ischemic necrosis [3]. In patients with metastatic bone lesions, adjuvant preoperative embolization facilitates surgical treatment to avoid intra-operative blood loss [4]. In inoperable patients, serial embolization has a palliative role, providing devascularization and pain relief with a successful outcome in up to 90% of cases [5–7]. However, a long-term efficacy of the treatment is limited due to the residual viability of tumor cells followed by disease reactivation so that multiple procedures are frequently necessary. The identification of biological markers to help in monitoring the treatment efficacy might facilitate the patient management, but no studies on this issue are available so far. The first step to achieve this goal is to select markers that significantly change after an effective embolization. The development and growth of metastatic lesions are continuously fueled by a vicious cycle that arises between metastatic cells, host stromal cells, and endothelial cells, leading to a production of angiogenic and bone-resorbing factors [8]. Therefore, modulation of the circulating levels of these factors could be informative of the response to embolization. Vascular Endothelial Growth Factor (VEGF-A) and basic Fibroblast Growth Factor 2 (FGF-2) are two major pro-angiogenic factors secreted by tumor cells [9]. VEGF-A induces cell proliferation, sprouting and tube formation of endothelial cells, increases vascular permeability, and supports invasion of stromal cells into the tumor [10]. FGF-2 potently promotes angiogenesis through a paracrine and autocrine mitogenic activity in endothelial cells [11, 12]. Moreover, this factor acts synergistically with VEGF-A in promoting angiogenesis, and its release has been associated with angiogenic switch in cancer development [13]. Both VEGF-A and FGF-2 have been widely studied for their clinical relevance in the circulation and considered as indicators of tumor recurrence and survival in patients with primary and metastatic carcinoma [14], as well as markers of treatment monitoring [15–17]. Hence, the development of pharmacological approaches

to inhibit the VEGF-A axis or, in the case of resistance to anti-VEGF-A therapy, to block the FGF-2 pathway, provided clinical benefit in several carcinomas [18–20]. The active isoform 5b of tartrate-resistant acid phosphatase (TRACP5b) has been identified as a specific marker of osteoclast activity [21]. Increased serum levels of this enzyme have been correlated with the presence of several conditions associated with increased bone resorption, including metastatic bone disease [22, 23]. Like VEGF-A and FGF-2, the circulating levels of TRACP5b have been suggested for treatment monitoring also in patients with bone metastases [24].

In this study, we investigated the modulation of the circulating levels of VEGF-A and FGF-2, as related to tumor angiogenesis, and of TRACP5b as related to bone resorption, in order to evaluate if the above markers change significantly after embolization of osteolytic metastases.

Patients and methods

Case series. Patients with clinical evidence of skeletal metastasis from carcinoma were enrolled in a prospective study approved by the institutional review board. Inclusion criteria were the presence of single, untreated osteolytic metastasis and the absence of primary tumors as verified by total body CT scan performed one week before selective arterial embolization. Exclusion criteria were surgical excision of the primary tumor within the previous 28 days, or the presence of multiple metastases at the time of embolization.

Embolization technique and radiological evaluation. Arterial selective embolization was performed as previously described [7]. Diagnostic digital subtraction angiography (contrast media iomeprol 300 mg/mL [Iomeron; Bracco, Milan]) and iohexol 350 mg/mL (Omnipaque; GE Healthcare, Milan, Italy) was performed before embolization to identify the feeding vessels. In all patients, angiography and selective arterial embolization were performed under local anesthesia using the Seldinger technique through femoral artery transarterial catheterization. Once the diagnostic angiogram was performed, the various feeding vessels were identified and superselectively catheterized with 4 or 5 French diagnostic catheters and microcatheters, and occluded using *N*-2-butyl cyanoacrylate (NBCA) as embolic agent (Glubran 2; GEM, Viareggio, Italy) in 33% Lipiodol (1 flacon [10 mL], Lipiodol Ultrafluido; Guerbet SpA, Genoa, Italy) 'sandwiched' with 5% glucosate solution to prevent polymerization with blood until administration of the embolic agent through the catheter. NBCA, 1 mL, was mixed with 33% Lipiodol, 2 mL. From the mixture, 1 mL was aspirated in an insulin (1 mL) syringe; depending on the pathologic vasculature, 0.1–0.2 mL of the aspirate mixture was injected 'sandwiched' with 2 mL of 5% glucosate solution under fluoroscopic control. Embolization was considered technically successful when there was stasis of the intravascular (IV) contrast material or elimination of tumor pathologic vasculature more than 90% compared with the initial diagnostic angiogram (visual scale). After 3 months, a clinical examination and a standard XRay were performed in order to assess the morphological reduction, stability or progression of the lesion; then a CT scan with IV contrast after 6 months. Moreover, embolization procedure was considered as clinical effective when the metastatic pain was reduced.

Immunoenzymatic assays. Peripheral venous blood was collected in Vacutainer tubes containing sodium citrate or without additive (Becton Dickinson, Plymouth, UK) before embolization (baseline) and 1, 3, and 6 months after the treatment. Within 1 hour, the blood samples were centrifuged at 2000 g for 10 minutes, followed by collection of plasma or serum which were aliquoted and stored at –70 °C until analysis.

Plasma levels of VEGF-A and FGF-2 were determined using commercial enzyme-linked immuno-sorbent assay kits following the manufacturer's instruction (Human FGF basic Quantikine HS ELISA Kit; catalog number HSFB00D; Human VEGF Quantikine ELISA Kit; catalog number DVE00; R&D Systems, Minneapolis, MN). The FGF-2 standard reference curve was prepared using protein concentrations between 0.3–20 pg/mL, and the sensitivity of the plasma samples was set by manufacturer to 0.07 pg/mL. The VEGF-A standard curve included concentrations between 31.2–2000 pg/mL and the sensitivity was 5 pg/mL. Serum TRACP5b was determined by a commercial solid-phase, immunofixed, enzyme activity assay (SBA-Sciences, Oulu, Finland). A standard reference curve was prepared using recombinant human TRACP5b between 0 and 10.3 U/L. Each sample was analyzed in duplicate and the optical density of each well was determined within 30 minutes, using a microplate reader set to 450 nm (Infinite 108 F200pro, Tecan, Milan, Italy) provided by a computer software capable of generating a curve fit and to determine the analyte concentration by interpolation. A linear regression model was applied to calculate the concentration of each sample, and with certain restrictions, automatically the software extrapolated also values outside the standard curve. Concentrations lower than 10% of test sensitivity are accepted, provided that the R of linear regression is higher or equal to 99%. Therefore, detection limits were set to 0.05 pg/mL for FGF-2, 4.5 pg/mL for VEGF-A, and 0.4 U/L for TRACP5b. As reference values we considered the 2.5th and the 97.5th percentile calculated in a population of healthy adults, according to our experience (TRACP5b 2.12 and 3.95 U/L) [25] or based on literature data (FGF-2 under detection limit and 6.4 pg/mL; VEGF-A 14 and 80 pg/mL) [26].

Statistical analysis. Nonparametric statistical analyses were performed with the StatView 5.0.1 software (SAS Institute Inc., Cary, NC). Cumulative data were expressed as arithmetic mean plus and minus the standard error of the mean (SEM). In order to impute a quantitative value, results of immunoenzymatic analysis below the detection threshold were considered arbitrarily as log-1 of the above mentioned limits. A nonparametric paired analysis (Wilcoxon signed

rank test) was applied to highlight significant changes between baseline marker levels and post-treatment values. The Wilcoxon analysis was combined with the Monte-Carlo method (XLStat, Addinsoft, NY, USA), which is a computational technique based on a random resampling of the data. P values were calculated on 100,000 simulations randomly generated, and all the statistical differences calculated by Wilcoxon analysis were confirmed with a confidence interval >99%. The Spearman rank correlation was used to evaluate the association between the markers. P-values <0.05 were considered as statistically significant.

Results

Seven patients undergoing arterial embolization of single osteolytic bone metastasis met the inclusion criteria and were enrolled in the study. The median age was 68 years (range, 62–82), and there were 3 males (43%) and 4 females (57%). Subject characteristics are shown in Table 1. The original primary carcinomas were kidney, breast, lung and thyroid cancers. According to the CT scan, 2 patients showed metastases >3 cm and <5 cm (medium size), whereas those >5 cm were classified as large size (mean metastasis size was 6.7±0.9 cm – range 4–10 cm). All embolization procedures were technically successful; selective catheterization and embolization of the feeding vessels was achieved in all cases, and in 7/7 cases post-procedural angiography showed interruption of the blood supply and more than 90% devascularization of metastases compared with diagnostic angiography (visual scale). All patients with the exception of patient 6 had a good pain response (Figure 1).

Radiological imaging at 3 and 6 months allowed to highlight morphological changes, i.e. reduction, stability, or increase in size of bone metastasis, as well as some information about the entity of bone remodeling. A significant size decrease (40%) was found in patient #2, along with areas of calcification and bone remodeling. In 5/7 individuals, a slight response (20% size reduction) with some little bone remodeling for more than 6 months was observed, while in one patient the size was unchanged. Side effects associated with embolization were not detected. Generally, adverse events are rare and include accidental embolization into non-tumor vessels, pseudoaneurysm of the femoral artery at the site of transarterial catheterization, pain due to ischemic necrosis of the tumor and post-embolization syndrome (symptoms such as fever, pain, and malaise).

Table 1. Characteristics of patients with osteolytic bone metastases, which were subjected to arterial embolization.

Patient number	Gender	Age (years)	Primary tumor histotype	Metastasis size (cm)
1	M	82	Renal carcinoma	6
2	M	63	Renal carcinoma	10
3	M	70	Thyroid carcinoma	10
4	F	63	Breast carcinoma	4
5	F	63	Lung carcinoma	4
6	F	62	Breast carcinoma	7
7	F	78	Renal carcinoma	6

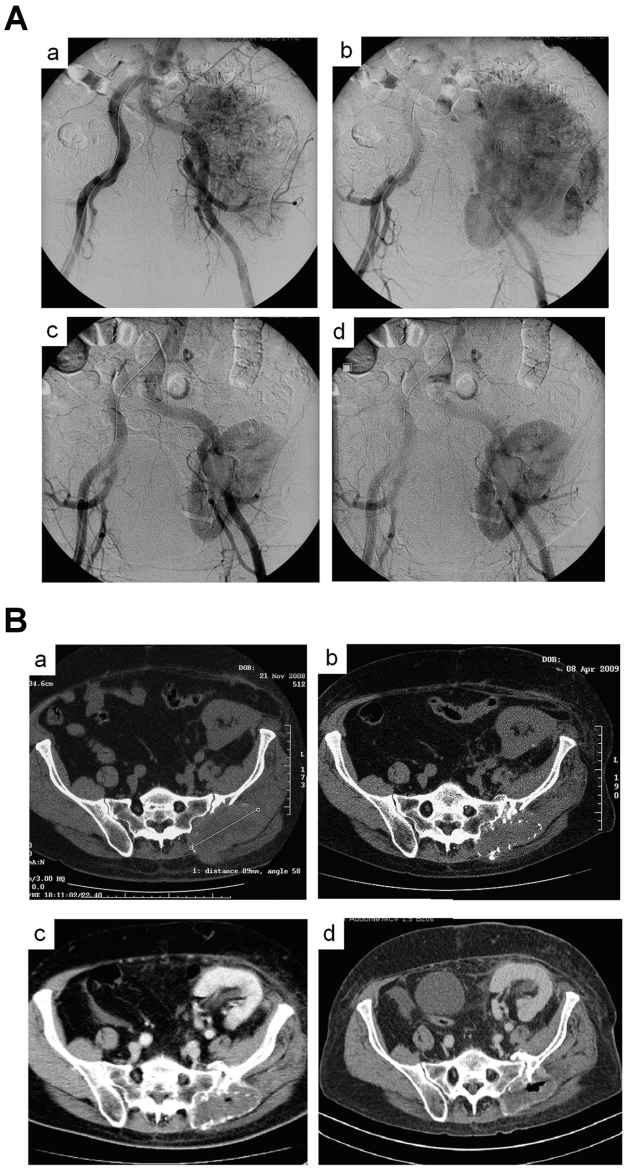

Figure 1. Clinical evaluation of embolization. (A) 63-year-old man with a pelvic renal cell carcinoma metastasis (renal transplantation in the left iliac fossa). Early (a) and late (b) phase digital subtraction aortography shows a hypervascular lesion in the left pelvis. The feeding vessels originate from the superior gluteal artery and ileo-lumbar artery. Late phase digital subtraction aortography after embolization shows complete occlusion of feeding vessels to the hypervascular lesion, with visualization of kidney transplantation (c–d). (B) Axial CT scan before embolization showing metastatic lesion of the left pelvis (a). At 6, 12, and 18 months (b–c–d), axial CT scan demonstrates peripheral ossification and tumor size reduction.

Modulation of VEGF-A, FGF-2, and TRACP5b levels after embolization. Plasma and serum samples were obtained before embolization and after 1, 3, and 6 months in all patients, with the exception of patient 5 at month 3.

The mean pre-embolization levels of VEGF-A were comparable to the values reported for healthy controls. A peak of VEGF-A production was observed 3 months after embolization for patients 4 and 7, and after 6 months for patient 1. In the other four patients, a small post-embolization decrease of plasma VEGF-A was detected (Table 2). However, there was no variation of VEGF-A levels from the baseline, and a very high variability was observed (Figure 2A).

Plasma FGF-2 levels were generally higher than those of the healthy population reported in the literature. Specifically, three of the seven patients had high levels of pre-embolization plasma FGF-2 that reverted to normal levels after the treatment (Table 2). When the trend in the whole group was analyzed, a significant decrease of FGF-2 values after embolization was observed, and this condition was maintained until month 6 (Figure 2B; p=0.028 at month 1, p=0.046 at month 3 and p=0.046 at month 6).

The serum levels of TRACP5b were comparable to those reported for healthy controls. The trend was constant over time for all the patients, with the exception of patients 6 and 7 which showed a marker decrease after 3 months (Table 2). After embolization, there was no reduction in the mean TRACP5b levels during the first month post-embolization, but a trend of decrease was observed in the following months (Figure 2C). Finally, the Spearman analysis demonstrated that FGF-2 had a partial negative correlation with TRACP5b (R=–0.405; p=0.0387), while no significant relationship between circulating markers and radiological finding has been found.

Discussion

The main purpose of arterial embolization is the induction of tumor devascularization, necrosis, and volume reduction by the voluntary occlusion of the most part of feeding vessels through the insertion of embolic agents [3]. It has been well documented that in highly vascular metastatic bone disease, preoperative embolization may represent an adjuvant to surgery for the control of intra-operative blood loss, thus facilitating tumor mass excision [4, 27, 28]. On the other side, palliative embolization ensures pain reduction in patients unsuitable for surgery [7, 29, 30]. In the case of serial embolization, monitoring of treatment efficacy through adequate biomarkers might help in the establishment of the most appropriate time for the re-intervention and to avoid unnecessary treatments. In this study, we focused on the analysis of some circulating markers related to tumor growth and osteolytic process. Our goal was to highlight significant changes that could be detectable after embolization of single bone metastasis, and to be more confident that such changes are related to this procedure we only included patients in

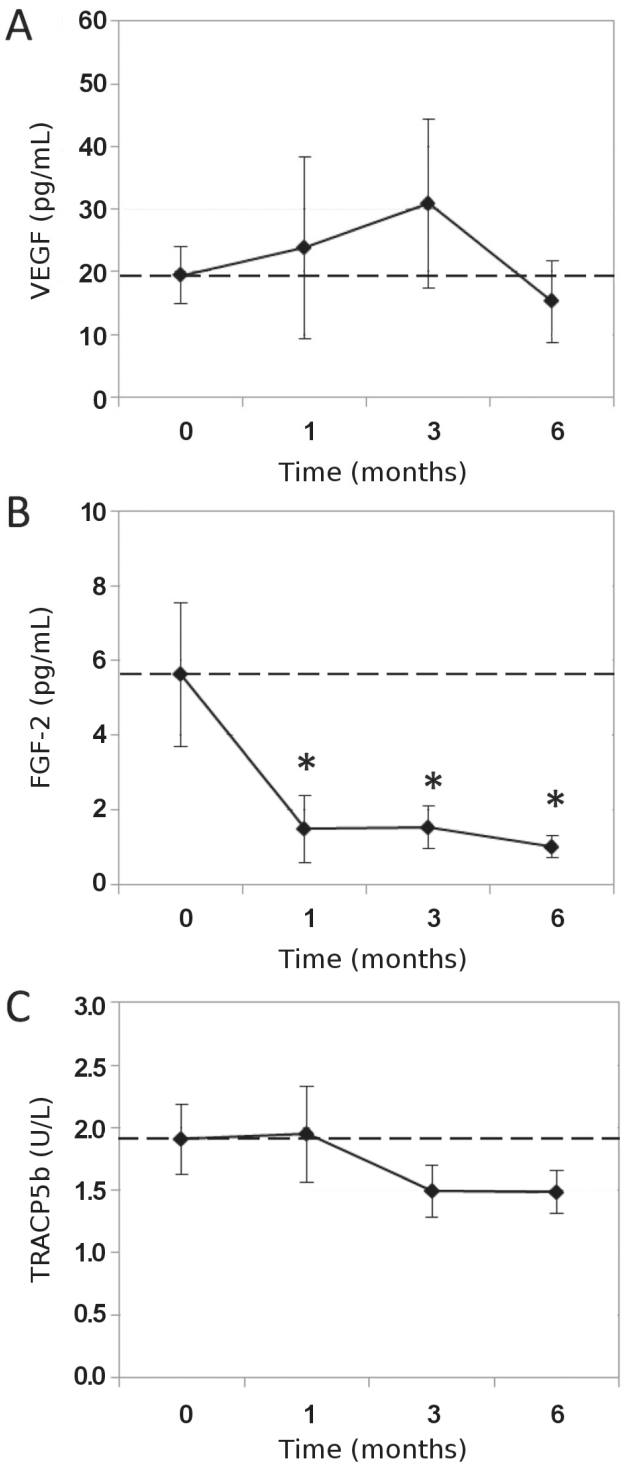

Figure 2. Analysis of marker following embolization. (A) Plasma VEGF-A detection by an immunoenzymatic assay before embolization (month 0) and 1, 3, and 6 months after the treatment, showing no difference from the baseline at month 0. (B) Plasma FGF-2 detection by an immunoenzymatic assay before embolization (month 0) and its significant decrease 1, 3, and 6 months after the treatment. *p<0.05. (C) Serum TRACP5b was detected by an immunoenzymatic assay before embolization (month 0) and 1, 3, and 6 months after the treatment, and was not affected by the treatment.

Table 2. VEGF, bFGF and TRACP5b levels before embolization (0) and 1, 3, and 6 months after the treatment.

	VEGF (pg/mL)				FGF-2 (pg/mL)				TRACP5b (U/L)			
Month →	**0**	**1**	**3**	**6**	**0**	**1**	**3**	**6**	**0**	**1**	**3**	**6**
#1	u.d.l.	u.d.l.	u.d.l.	50.139	1.341	1.639	0.381	0.464	1.849	1.83	1.659	1.402
#2	27.943	4.503	17.654	19.548	2.483	1.523	3.179	1.109	1.37	1.225	1.316	1.955
#3	18.546	4.656	6.205	10.537	1.689	0.05	0.281	2.036	2.135	1.692	1.652	1.431
#4	30.476	53.812	80.81	25.374	13.995	6.672	2.897	1.573	0.702	0.849	0.897	0.809
#5	12.34	u.d.l.	n.a.	u.d.l.	9.95	0.331	n.a.	u.d.l.	1.856	1.764	n.a.	1.882
#6	34.435	4.769	17.295	20.25	8.427	0.199	2.252	0.315	2.395	2.308	1.081	0.999
#7	12.34	98.961	63.386	5.88	1.474	u.d.l.	0.149	1.473	3.045	3.994	2.348	1.905

n.a. = not available; u.d.l. = under detection limit

whom the primary tumor had been excised. Conversely, the tumor histotype was not considered, since we aimed to identify markers that change significantly and stably irrespective of the characteristics of the primary source.

Neoangiogenesis is an essential condition for tumor initiation and progression [31]. Among the most investigated angiogenic factors, both VEGF-A and FGF-2 play a major role in the pathological growth of blood vessels, and several studies support their prognostic and clinical implications in a variety of cancers [10, 14, 32]. In osteolytic bone metastases, the angiogenic and bone resorption mechanisms are closely related. It has been shown that VEGF secreted by neoplastic cells enhances bone resorption mediated by osteoclasts [33] and that the inhibition of angiogenesis reduces the growth of carcinoma cells within the bone [34]. We therefore sought a possible correlation between neoangiogenesis and osteolysis, as expressed by circulating VEGF-A, FGF-2, and TRACP5b, and the success of embolization, hypothesizing that the blood concentration of these markers is correlated to the maintenance of the vascular occlusion and to the lytic activity of metastases. We quantified VEGF-A levels in plasma, since it is considered a better indicator of tumor progression than serum VEGF-A, which is significantly affected by the contribution of VEGF-A released by α-granules during platelet activation [26]. In our case series, VEGF-A levels at baseline were not increased when compared to values referable to healthy control [25, 26]. After embolization, a slight tendency of decrement in four of the seven patients was detected, but we do not have enough information to directly correlate this trend to the treatment. Similarly, the serum levels of TRACP5b also resulted to be close to normal limits [25]. Only a small trend of decrease was detectable starting at month 3, perhaps as an indirect effect due to the blockade of osteoclast stimulation by tumor cells consequent to the embolization. We were not surprised to find very low levels of these markers. In our series, metastasis was a local phenomenon, and the absence of the primary tumor that substantially contributes to the release of these markers may possibly explain this phenomenon. Moreover, VEGF-A is not necessarily a good indicator of cancer progression [35] as it is elevated only early in the clinical course [36]. With respect to TRACP5b, its activity might be elevated only in patients with multiple bone metastases rather than in all metastatic patients [22, 37], suggesting its limited utility as a marker for the presence of metastases. On the contrary, in our series the FGF-2 levels tended to be higher than reported in the literature for healthy individuals [17, 26]. Deregulation of FGF signaling in neoplasm development continues to emerge [38] and the role of circulating FGF-2 in clinical tumor progression has been strongly suggested [39]. However, its direct involvement in bone metastasis activity has never been described. Beyond its well-documented potent pro-angiogenic activity, FGF-2 plays an essential role in bone homeostasis [40]. In particular, it has been reported to stimulate osteoclastogenesis and bone resorption via the activity on precursors or a direct action on mature osteoclasts [41]. Furthermore, it is produced by osteoblasts during bone formation and entrapped in the newly synthesized bone matrix [42]. In this context, we can speculate that the tendency of increase of plasma FGF-2 is possibly derived both from the production by tumor cells and the release from the bone microenvironment after matrix degradation. However, we were surprised to find a prompt and significant post-embolization FGF-2 decrement that persisted for 6 months. Since all the embolizations were technically successful, this reduction might optimistically reflect the maintenance of vascular occlusion, and, ultimately, could be predictive of a favorable outcome. FGF-2 levels were negatively correlated to those of TRACP5b, possibly due to the presence of a negative feedback. As mentioned above, FGF-2 is abundant in the bone tissue and can exert a differential action on skeletal metabolism. In particular, FGF-2 can directly hamper osteoclastic activity – via the blockade of M-CSF signaling or the alteration of the cytoskeleton organization – or it can indirectly inhibit their activity by a feedback mechanism subsequent to its release during bone degradation followed by the activation of osteoblastogenesis [41, 43–45].

The major limitation of this pilot study is the small number of cases. Patient recruitment was more difficult than expected since often the embolization is not considered as a first-line option for patients who have a single, untreated bone metastasis. However, the inclusion criteria allowed to exclude that changes in circulating factors were determined by primary tumor and/or are a consequence of a large skeletal involvement. Even though the results cannot be used to draw inferences regarding the diagnostic performance of selected circulating markers, case-series studies can provide valuable information to test hypotheses or to observe a trend [46]. One the one hand, the lack of significant results in a small case series does not allow conclusions about the potential role of VEGF-A and TRACP5b for monitoring post-embolization. On the other hand, according to the primary objective of the study, we were able to identify a circulating marker that changes significantly and stably irrespective of the cancer type, thus suggesting that FGF-2 could be a suitable tool for monitoring the osteolytic lesions after arterial embolization. The clinical relevance of this promising marker has to be confirmed in "ad hoc" studies planned to accomplish specific objectives in a larger case series and over a longer period.

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