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Review

Bone turnover markers in serum and urine as diagnostic, prognostic and monitoring biomarkers of bone metastasis

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ABSTRACT

Bone metastases are characterized by increased osteoblastic and/or osteolytic processes depending on the tumor type. The altogether destructive effect of metastasis formation promoted by increased metabolic activity raises the release of components from the osseous metabolism into the blood stream. These components are either enzymes directly involved in the alteration processes, metabolites/proteins that develop during this or bone matrix proteins released during this. These biomarkers are categorized in relation to their involvement in the bone formation or resorption as bone formation and resorption markers. Based on a PubMed literature search, a critical appraisal of the various biomarkers for diagnostic, prognostic, and monitoring purposes is given for patients with skeletal metastases caused by breast, prostate, lung, or renal cell carcinomas.

Contents

Abbreviations: BAP, bone-specific alkaline phosphatase; BCE, bone collagen equivalents; BSP, bone sialoprotein; CLIA, chemoluminescence immunoassay; CREA, urinary creatinine; CTX, carboxy-terminal cross-linking telopeptide of type I collagen; DPD, deoxypyridinoline (lysylpyridinoline); DKK-1, Dickkopf-1; ECLIA, electrochemoluminescence immunoassay; ICTP, carboxy-terminal cross-linking telopeptide of type I collagen, generated by MMPs; NTX, N-terminal cross-linking telopeptide of type I collagen; OC, osteocalcin; OPG, osteoprotegerin; OPN, osteopontin; PICP, carboxy-terminal propeptide of type I procollagen; PINP, amino-terminal propeptide of type I procollagen; PYD, pyridinoline (lysylhydroxypyridinoline); RANKL, receptor activator of NF-kappaB ligand; RIA, radioimmunoassay; ROC, receiver-operating characteristics; TRAP5b, tartrat-resistant acid phosphatase type 5b

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1. Introduction

Metastases in the bone lead to defective hemostasis that physiologically exists in the balance between the formation of new and the resorption of old osseous segments by osteoblasts and osteoclasts, respectively [1]. The morbidity of patients with advanced cancer is essentially caused by the occurrence of bone metastases [2]. Most patients with bone metastases experience complications, the so-called skeletal-related events that summarize hypercalcemia, severe bone pain, pathological bone fractures, spinal cord compression, and surgery to bone because of bone instability. Thus, an early diagnosis and specific prediction of patients at risk of skeletal complications is of great interest to improve the clinical management of these patients not only in reducing these complications but also increasing overall survival by a bone-targeted therapy [3–5].

The histomorphology of bone metastases showed metastasis formation in form of increased osteolytic and/or osteoblastic processes depending on the tumor type. During the metastatic process, components from the osseous metabolism are increasingly released into the blood stream, promoted by increased metabolic activity and the altogether destructive effect of metastasis formation [6]. These components are either enzymes directly involved in the alteration processes, metabolites that develop during this or bone matrix proteins released during this [7]. These biomarkers are generally categorized into bone formation and resorption markers based on their reflection of osseous formation or resorption [8]. Their determination in the serum and/or urine provides the opportunity to use them for questions in diagnostics, evaluation of the prognosis and treatment of patients with skeletal metastases [9]. In this respect, the aim of this review is focused on the appraisal of the current usefulness of these markers in practice rather than discuss the molecular processes in detail. There is the intention to sensitize basic scientists for the future-oriented task to translate novel molecular findings in bone metastatic processes into improved clinical tools.

2. Bone formation and resorption markers and their determinations in serum and urine

2.1. Analytical methods and variability of bone markers

A number of bone markers can be determined using commercial tests in the meantime. In regards to the method, enzyme immunological procedures have established themselves over radioimmunoassays. These tests are increasingly adapted using laboratory machines that achieve higher analytical reliability during the determination as compared to manual ELISA methods [10,11]. Serum/plasma is recommended as test material over urine for reasons of better practicability and lower inter- and intra-individual variability [12,13]. Sample collection and handling are essential pre-analytical factors that could differently affect the stability of the different bone markers [14,15]. As the biological variability of bone markers essentially influences their clinical interpretation, the influencing factors of this variability have to be regarded [16,17]. Age, sex, or menopausal status are uncontrollable factors and should be considered in form of different reference intervals (see also Table 1). In contrast, controllable factors of the biological variability and the pre-analytic phase (e.g., diurnal, seasonal, menstrual, diet, and exercise effects; kind of samples, storage) could be accounted to a great extent by a standardized sampling process (time and conditions of sampling, subsequent processing of samples) [15,16].

Table 1 shows test systems with their orientating reference ranges for the most commonly used markers [18–20]. This assay overview is necessary since bibliographical information on the test systems and their manufacturers to date are often no longer applicable due to company takeovers. The significant problem of method comparability of bone marker determinations is made clear by the sometimes significant differences in the reference intervals and the different reference systems used for the same markers.

Sex- and age-dependent reference intervals that were calculated based on determinations using a numerically sufficient reference population in consideration of recognized statistical procedures have been rare to date [18,21]. This problem was recently discussed in detail for the three bone markers bone-specific alkaline phosphatase (BAP), amino-terminal propeptide of type I procollagen (PINP), and carboxy-terminal cross-linking telopeptide of type I collagen (CTX) [18]. The comparability of the results of bone marker determinations between laboratories is also unsatisfactory even if identical methods were used [10,22]. This lack of comparability of bone marker determinations has already been recognized in osteoporosis diagnostics [23–25]. The goal is to harmonize the methods and use joint standards for the analyte measurements, but also include pre- and post-analytical aspects [15,26]. First efforts have already been made in this regard for skeletal metastasis diagnostics [27].

2.2. Biomarkers of bone formation

2.2.1. Bone-specific alkaline phosphatase

Bone-specific **a**lkaline **p**hosphatase (BAP) promotes bone mineralization. The enzyme is secreted by the osteoblasts. Increased concentration in the serum is primarily considered a sign of primarily increased osteoblast activity or secondarily as a corrective reaction as a result of increased bone resorption [7]. Preceding chemical and electrophoretic methods as selective approaches to discriminate BAP from the liver and intestinal alkaline phosphatase isoenzymes have been replaced by immunological methods [28]. There are two available methods which measure either the protein mass (e.g., Access Ostase) or enzyme activity (e.g., Ostase BAP EIA) as manual and automated test procedures (Table 1). Both methods provide highly comparable results [29].

2.2.2. Osteocalcin

Osteocalcin (OC) is the dominant non-collagenous protein of the bone matrix [30]. This bone-specific protein is synthesized by osteoblasts depending on vitamins K and D3. Various osteocalcin fragments

are detected in the blood [31]. However, the intact molecule of 49 amino acids is subject to quick degradation in circulation and in vitro due to proteolytic cleavage between amino acids 43 and 44 [30,31]. Recently used methods, the so-called N-MID osteocalcin assays (see Table 1), focus on the determination of the individual fragments, such as the mostly stable N-terminal N-MID fragment from amino acids 1–43 [32,33]. These assays utilize two monoclonal antibodies, one specific for epitopes on the N-MID fragment and the other specific for the N terminal OC fragment. Thus, both the intact OC and the N-mid fragment are detected and robust assays under routine conditions are generally ensured [34].

2.2.3. Propeptides of type I procollagen

Type I collagen as the significant part of the organic bone matrix develops from type I procollagen. During this, propeptides are separated by proteases on the N- and C-terminal ends of the procollagen [35]. They reach the bloodstream and can be assessed as biomarkers for the synthesis of the bone matrix. Corresponding to their origin, the propeptides are called C-terminal or N-terminal propeptides of type I procollagen (PICP; PINP). PINP occurs in the serum in two forms: as intact, trimeric peptide corresponding to the native separation product of procollagen during the synthesis of type I collagen and as monomeric peptide which is rather a degradation product of procollagen [35–37]. In contrast to this view, there has been the assumption that the released intact PINP is rapidly degraded to the monomeric form when it is released into circulation and would make it necessary to determine both forms [38]. Determinations of the intact, trimeric PINP and the total PINP in the sum of intact and monomeric PINP can, in the meantime, be performed on laboratory analyzers (Table 1). With a few exceptions, such as patients with kidney insufficiency due to a decreased clearance of the monomeric PINP form [39], the results of total PINP and intact PINP are well comparable [37,40]. However, differences under various clinical conditions have to be considered [35].

2.2.4. Sclerostin and Dickkopf-1 as negative regulators

Sclerostin and the Dickkopf-1 protein (DKK-1) are recently discovered biomarkers that act through their inhibition of the Wnt signaling pathway as negative regulators on the osteoblast development and activity [41]. Sclerostin is mainly secreted by osteocytes and DKK-1 both by osteoctyes and osteoblasts, but also by non-osseous cells; and both proteins are detected in serum/plasma [42]. However, the peripheral concentrations of these markers under different clinical situations and treatment conditions do not always reflect the expected data. The relationship to the really observed bone mass and other circulating bone turnover markers has often been found contrary to their assumed regulative effect on osteoblasts [43–46]. Thus, the influence of biological factors (age, menopausal and hormonal status, renal function) on the concentration of both analytes in serum/plasma [47,48], the still not fully understood molecular processes in the bone [49,50], but also simply unresolved analytical problems [51] may currently be reasons for these substantial interpretation difficulties of the two analytes in serum/plasma. For example, the three commercially available sclerostin ELISAs (Mesa Scale discovery, Rockville, Maryland, USA; TECOmedical, Sissach, Switzerland; Biomedica, Wien, Austria) resulted, despite a reasonable correlation of data, in up to 10- to 30-fold different mean concentrations [44]. This insufficient comparability and traceability of data seems mainly caused by the different detection of the fragments or the full protein by the three immunoassays. It is perhaps a little like the situation of the initial osteocalcin measurements. In case of DKK-1, its general effect in tumorigenesis has to be considered [49]. As DKK-1 is not exclusively expressed in bone but also in other organs, different changes of circulating DKK-1 levels were observed in various cancer entities without evidence of a direct osseous involvement [52,53]. For example, patients suffering from lung cancer or melanoma showed distinctly increased serum concentrations of DKK1 compared with controls while significantly decreased concentrations were found in patients with gastric, colorectal, ovarian, and cervical cancer [52,53]. However, in contrast to these data, another working group described significantly increased concentrations in gastric and cervical cancer [54]. All these uncertainties require a particular caution in interpreting the concentrations of both circulating DKK-1 and sclerostin. Therefore, we deliberately refrained to include the above mentioned methods with their suggested reference intervals into Table 1. However, we are certain that it is worth evaluating especially the prognostic, predictive, and monitoring potential of both markers in future studies under the condition that the analytical issues will be resolved.

2.3. Biomarkers of bone resorption and osteoclastogenesis

2.3.1. Pyridinoline and deoxypyridinoline

The helix shape of bone collagen is stabilized by crosslinks of lysine or hydroxylysine residues. These components called pyridinoline (PYD) and deoxypyridinoline (DPD) are released in bone resorption and eliminated renally without further metabolization [8]. DPD is almost solely found in the bone and is thus a specific indicator of bone structure as PYD that is also found in cartilage. In urine, approximately 40% of the pyridinoline crosslinks are eliminated as free components, the rest as peptide-bound forms [55,56]. There are methods available for the determination of free as well as total crosslinks (sum of total and peptide-bound crosslink components) in the urine and the serum (Table 1).

2.3.2. Cross-linked telopeptides of type I collagen

Type I collagen releases carboxy- as well as amino-terminally cross-linked telopeptides (abbreviations: CTX and NTX) during bone resorption into the blood stream [57]. CTX and NTX can be measured in the serum and the urine with manual and automated tests. CTX peptides occur in heterogeneous forms; they occur as native, non-isomerized αCTX and isomerized βCTX form. βCTX has a significantly circadian rhythm depending on the eating, for which reasons blood tests for determination of βCTX should be determined after having fasted in the early morning [58]. αCTX reflects the short collagen half-life in osseous metastases better than βCTX [59]. This probably explains the higher discrimination ability of αCTX tests in the diagnostics of osseous metastases in comparison to the βCTX analyses performed much more commonly to date. However, αCTX can to date only be determined in the urine [59].

ICTP (carboxy-terminal cross-linking telopeptide of type I collagen) is a specific telopeptide that is separated from collagen by metalloproteinases. It is a trivalent cross-linked telopeptide of 8.5 kD with two phenylalanine-rich domains between the two α1 collagen chains.

2.3.3. Tartrate-resistant acid phosphatase 5b

Osteoclasts contain the tartrate-resistant acid phosphatase, type 5b (TRAP5b) as bone-specific isoenzyme. The enzyme is released into the bloodstream during bone resorption. It can be determined in the serum with a specific ELISA (see Table 1). The concentration in the serum is determined by the number and activity of the osteoclasts involved in the resorption process and thus generally reflects the extent of bone resorption [60]. However, studies with a cathepsin K inhibitor showed a discordant behavior between the decrease of the typical resorption markers CTX and NTX in comparison to the increase of serum TRAP5b [61]. This phenomenon has been interpreted as treatment effect resulting in an increased number of osteoclasts but a decreased cellular resorption process due to the cathepsin K inhibition [62].

2.3.4. Bone sialoprotein and osteopontin

The bone sialoprotein (BSP, bone sialoprotein 2) and osteopontin (OPN, bone sialoprotein 1) are assigned to the proteins of the SIBLING (small integrin-binding ligand, N-linked glycoprotein) family today. The conventional abbreviations BSP and OPN are used in the following

Table 1
Reference intervals and assays of bone turnover markers
The reference intervals ([a]95%, [b]90% reference interval, [c]without exact definition) were taken from the instructions manuals of the test kits, calculated as 95% reference intervals using the data given in these manuals or were taken from literature, indicated by a corresponding reference.

BONE FORMATION MARKER

Analyte	Sample	Assay/principle	Producer	Reference intervals
BAP	Serum Plasma	Access ostase/CLIA, automated	Beckman Coulter, Brea, CA, USA	Women, premenopausal[a], 3.2–18.8 μg/L Women, postmenopausal[a], 5.3–22.7 μg/L Men[a], 5.0–22.8 μg/L
		IDS-iSYS Ostase BAP/CLIA, automated Ostase BAP EIA/ELISA	IDS Inc., Scottsdale, AZ, USA	Women, premenopausal[a,][18], 6.0–22.7 μg/L Women, postmenopausal[a,][18], 8.1-31.6 μg/L Men (>45 years)[a,] [18] 7.5-26.4 μg/L as Access ostase assay
		MicroVue BAP/ELISA	Quidel Corp., San Diego, CA, USA	Women, premenopausal[b], 11.6-29.6 U/L Women, premenopausal[b], 14.2–42.7 U/L Men[b], 15.0–41.3 U/L
OC	Serum Plasma	N-MID osteocalcin/ECLIA, automated	Roche Diagnostics, Mannheim, Germany	Women, premenopausal[b], 11–43 μg/L Women, postmenopausal[b], 15–46 μg/L Men (>50 years)[b],14–46 μg/L
		N-Mid Osteo-calcin/ELISA IDS-iSYS N-MID Osteocalcin/CLIA, automated	IDS Inc., Scottsdale, AZ, USA	Women, premenopausal[a], 12.8–55.0 μg/L Women, postmenopausal[a], 8.4–33.9 μg/L Men[a], 9.6–40.8 μg/L
		MicroVue Osteocalcin/ELISA	Quidel Corp., San Diego, CA, USA	Adults (>25 Jahre)[c], 3.7–10 μg/L
		Undercarboxylated Osteocalcin EIA Kit/ELISA	TakaRa Bio Inc., Shiga, Japan	ca. 20% of total OC in serum; no further data
PICP	Serum Plasma	MicroVue CICP/ELISA	Quidel Corp., San Diego, CA, USA	Women, postmenopausal[b], 69–147 μg/L Men (>25 years)[b], 76–163 μg/L
		Procollagen Type I C-Peptide (PIP)/ELISA	TakaRa Bio Inc., Shiga, Japan	Adults[a], 161–757 μg/L
PINP	Serum Plasma	UniQ PINP/RIA	Orion Diagnostica Oy, Espoo, Finland	Women[c], 19–83 μg/L Men[c], 22–87 μg/L
		IDS-iSYS Intact PINP/CLIA, automated	IDS Inc., Scottsdale, AZ, USA	Women, premenopausal[a,][18] 19.3–76.3 μg/L Women, postmenopausal[a,][18], 18.2–102.3 μg/L Men (>45 years)[a,][18], 19.1–77.0 μg/L
		Total P1NP/ECLIA, automated	Roche Diagnostics, Mannheim, Germany	Women, premenopausal[a], 13.8–60.9 μg/L Men[a], 13.9–85.5 μg/L

Bone resorption and osteoclastogenesis marker

Analyte	Sample	Assay	Producer	Reference interval
DPD	Urine	MicroVue DPD/ ELISA	Quidel Corp., San Diego, CA, USA	Women (25–44 years)[b], 3.0–7.4 nmol/mmol CREA Men (25–55 years)[b], 2.3–5.4 nmol/mmol CREA
Total DPD	Serum Urine	MicroVue Total DPD/ELISA	Quidel Corp., San Diego, CA, USA	Women, (25–44 Jahre)[a], 2.18–4.68 nmol/L Men (25–55 Jahre)[a], 1.95–4.54 nmol/L 19–325 nmol/L[c]
	Serum	MicroVue Serum PYD(only free)/ELISA	Quidel Corp., San Diego, CA, USA	Adults, 1.09–2.79 nmol/L[c]
	Urine	MicroVue PYD (free PYD + DPD)/ ELISA	Quidel Corp., San Diego, CA, USA	Women, premenopausal[b], 16.0–37.0 nmol/mmol CREA Men[b], 12.8–25.6 nmol/mmol CREA
CTX	Serum Plasma	ß-CrossLaps/ECLIA, automated	Roche Diagnostics, Mannheim, Germany	Women, premenopausal[a], 31–568 ng/L Women, postmenopausal[a], 113–999 ng/L Men (30–50 years)[a], 22–578 ng/L (>50-70 years), until to 692 ng/L (>70 Jahre), until to 855 ng/L
		CrossLaps Serum/ELISA	IBL, Toronto, Canada	Women, premenopausal[a], 112–738 ng/L Women, postmenopausal[a], 142–1351 ng/L Men (30–50 years)[a], 115–748 ng/L
		Serum CrossLaps (CTX-I)/ ELISA	IDS Inc., Scottsdale, AZ, USA	Women, premenopausal[a] [19], 0.39–3.20 nmol/L Women, postmenopausal[a,][19], 0.36–5.55 nmol/L
		IDS-iSYS CTX-I (CrossLaps)/ ECLIA, automated		Women, premenopausal[a] [18], 50–670 ng/L Women, postmenopausal[a] [18], 90–1050 ng/L Men (>45 years)[a,] [18], 90–730 ng/L
	Urine	CrossLaps Urine ELISA/ Urine BETA CrossLaps (CTX-I)/ ELISA	IBL, Toronto, Canada IDS Inc., Scottsdale, AZ, USA	Women, premenopausal[a], 67–544 μg/mmol CREA Women, postmenopausal[a], 121–874 μg/mmol CREA Men (31–80 years)[a], 54–559 μg/mmol CREA
		Alpha Crosslaps EIA/ELISA	IDS Inc., Scottsdale, AZ, USA	Women, premenopausal[a], 0.10–0.99 μg/mmol CREA Women, postmenopausal[a], 0.17–2.26 μg/mmol CREA Men[a], 0.13–1.13 μg/mmol CREA
NTX	Serum Plasma	Osteomark NTx Serum/ELISA	Alere Inc., Waltham, MA, USA	Women, premenopausal[a], 7.7–19.3 nmol BCE/L Men (31–80 years)[a], 8.1–24.8 nmol BCE/L
	Urin	Osteomark NTx Urine/ELISA	Alere Inc., Waltham, MA, USA	Women, premenopausal[a], 14–74 nmol BCE/mmol CREA Männer (31–87 Jahre)[a], 13–78 nmol BCE/mmol CREA
ICTP	Serum Plasma	UniQ ICTP/ELISA and RIA	Orion Diagnostica Oy, Espoo, Finland	Women[c], 1.6-4.2 μg/L Men[c], 1.5-4.3 μg/L
TRAP5b	Serum Plasma	MicroVue TRAP5B EIA/ELISA	Quidel Corp., San Diego, CA, USA	Women, premenopausal[a], 0.25-5.64 U/L Women, premenopausal, until to 9.12 U/L Men[a], 1.26–6.74 U/L
BSP	Serum Plasma	Bone Sialoprotein (BSP)/ELISA	Immun-diagnostik AG, Bensheim, Germany	Without data
OPN	EDTA-Plasma	Quantikine ELISA Human Osteopontin	R&D Systems, Minneapolis, MN, USA	Adults[a], 46–144 μg/L
OPG	Serum Plasma	Osteoprotegerin/ELISA	Immun-diagnostik AG, Bensheim, Germany	Adults[a] [20], until 3.60 pmol/L
RANKL	Serum Plasma	Total sRANKL/ ELISA	Immun-diagnostik AG, Bensheim, Germany	Women, premenopausal[a,][20], until 3.29 pmol/L Men[a,][20], until 1.66 pmol/L

and the current official abbreviations for BSP and OPN are the abbreviations IBSP (integrin-binding sialoprotein) and SPP1 (secreted phosphoprotein 1). Both proteins are components of the non-collagenous bone matrix. BSP is expressed in osteoclasts and osteoclast-like cell lines [63,64], but like OPN also in osteoblasts [65]; however, both also occur in other cells. The divergent change of serum BSP in comparison to alkaline phosphatase as bone formation marker and its parallel changes to other markers of bone resorption in metabolic and malignant bone diseases suggested to classify BSP as bone resorption marker [66,67]. For the sake of clarity we list both proteins in this category together, even though significant molecular biological findings suggest in the meantime that they themselves are involved in the regulation of bone resorption and formation as well as in important metastasis formation processes [68,69]. It has been shown that BSP and OPN are essential factors in bone metastasis of osteotropic cancers from breast, prostate, and lung [70]. Both markers can thus only be considered bone markers for osseous metastases with reservations.

BSP determinations were to date only possible with radioimmunoassay; in the meantime a commercial ELISA is also available for this (Table 1).

2.3.5. Osteoclastogenesis proteins: receptor activator of NF-kappaB ligand and osteoprotegerin

The transcription factor RANK (receptor activator of NF-kappaB) controls the differentiation and activation of osteoclasts together with its ligand (RANKL) and osteoprotegerin (OPG). The regulatory circuit of this triple system is disrupted when osseous metastases develop. Elevated OPG values in the serum of patients with osseous metastases of different tumors suggest this [71,72]. ELISAs for both analytes are available (Table 1). Circulating RANKL occurs in blood as free RANKL and OPG-sRANKL-complex. Using different measurement conditions, both the free and the so-called total sRANKL can be differentially measured. However, the low analytical sensitivity of the assay [20] and also the uncertainty whether free sRANKL or bound RANKL forms should be measured as relevant markers [73] has so far restricted the practical implementation of the assay.

3. Bone biomarkers in the clinic: application possibilities and problems

In the following we will initially discuss the general principles of the applications of bone markers. Afterwards we will discuss the specific problems in carcinomas that metastasize most commonly in bone (breast, prostate, lung, and renal cell carcinomas) based on bibliographic data.

The establishment of analysis methods for determination of specific bone markers in biological fluids began more than 25 years ago. Numerous clinical studies have been performed in the meantime in order to estimate their significance in diagnostics, evaluation of the prognosis and treatment of patients with osseous metastases. However, the expectations of being able to solve many open clinical questions by determination of these markers, resulting from the pathobiochemical background, have only been partially met. There are numerous reasons for this and this is still being researched.

3.1. Bone markers as diagnostic indicators

Patients with osseous metastases usually have elevated bone biomarker parameters compared to carcinoma patients without metastases. However, there is the current consensus that the diagnostic sensitivity and/or specificity of bone markers are not sufficient enough to utilize the results for metastasis diagnostics due to the high spread of the values as mentioned above [27]. This is also associated with the question whether bone markers can detect metastasis formation earlier than imaging procedures [74–76]. Thereby it should be borne in mind that this is an issue which arises time and time again since newly developed imaging procedures will surely replace the conventional bone scintigraphy as standard procedure for detecting bone metastatic lesions [77–79].

There are generally advantages to bone marker determinations compared to imaging procedures [80]. They are lower cost, exact quantitative information that is mostly independent of the processor and better differentiation between tumor progression and reactive processes in terms of wound healing as a result of treatment (flare phenomenon). However, there is little comparative data to date, since this requires long-term prospective examinations. For example, follow-up examinations of patients with breast carcinoma did show that the marker values often do not exceed the upper limit of the reference range in the formation of osseous metastases [81]. These results are thus interpreted as unremarkable if no reference to the original value can be made. Similar results were reported in lung cancer patients [82]. However, a great disadvantage is that the information on the bone markers' reference ranges is often only orientating [83,84] and does not generally consider the accepted guidelines for the determination of reference intervals [85,86]. It is also decisive in the evaluation of the diagnostic validity of bone markers that different patterns of markers in the serum/urine are detected in osseous metastases of the various primary tumors. It does not matter here whether the respective metastasis is primarily of osteoblastic or of osteolytic nature. It is important how the normal coupling between bone formation and bone resorption is disturbed. This coupling between these two processes is based on the interaction of the different cell types of the bone and its microenvironment including their different released soluble and membrane products [87]. The coupling processes can be differently disturbed by metastases derived from different cancer types with typical changes of the circulating bone turnover markers. For example, the formation as well as resorption markers in the serum are elevated in case of typical osteoblastic osseous metastases of the prostate carcinoma [88,89]. In contrast, the resorption marker values in the serum might be unchanged, e.g. in osteolytic osseous metastases characteristic in renal cell carcinoma, different from what was expected [90,91]. A comparison between bone markers in metastasized patients as well as between the different markers of a tumor entity and between the different tumors clarifies the problem (Fig. 1A-D). Furthermore, only a limited number of markers were determined in many studies so that a general conclusion regarding the "clinical utility of bone markers" is rather unwarranted. A statement on diagnostic validity can thus only be made for the markers used for the respective tumor and not bone markers in general. We will discuss this further in the following chapters on individual tumors.

3.2. Bone markers as prognostic indicators

Bone markers are increasingly being used to estimate the prognosis of patients with osseous metastases [113]. There are in the meantime extensive results of prospective and retrospective studies for individual tumor entities using various bone markers. The available literature here is much more precise and clear than in the diagnostic questions. This was also expressed in the consensus publication compiled by an international workgroup on the use of bone markers in malignant osseous lesions [27]. It is described here how this biomarker should be evaluated

Notes to Table 1

Abbreviations: BAP, bone-specific alkaline phosphatase; BCE, bone collagen equivalents; BSP, bone sialoprotein; CLIA, chemoluminescence immunoassay; CREA, urinary creatinine; CTX, carboxy-terminal cross-linking telopeptide of type I collagen; DPD, deoxypyridinoline (lysylpyridinoline); ECLIA, electrochemoluminescence immunoassay; ICTP, carboxy-terminal cross-linking telopeptide of type I collagen, generated by MMPs; NTX, amino-terminal cross-linking telopeptide of type I collagen; OC, osteocalcin; OPG, osteoprotegerin; OPN, osteopontin; PICP, carboxy-terminal propeptide of type I procollagen; PINP, amino-terminal propeptide of type I procollagen; PYD, pyridinoline (lysylhydroxypyridinoline); RANKL, receptor activator of NF-kappaB ligand; RIA, radioimmunoassay; TRAP5b, tartrat-resistant acid phosphatase, type 5b.

in regards to its prognostic validity according to conventional evidence criteria using the example of NTX determinations in the urine of patients with osseous metastases of various tumor entities. See further information in the paragraphs on individual tumors.

3.3. Bone markers in follow-up examination

The disadvantage to use bone markers as diagnostic indicators on the basis of population-based reference intervals is not only caused by the often poorly defined reference ranges as described above. Another main reason is the large variability of various bone markers between individuals resulting in wide reference ranges. This variability cannot always be compensated by the establishment of age- or sex-specific reference intervals. However, it is important for the clinician to know which change of bone marker concentrations is statistically significant. Serial measurements that are generally performed in the follow-up of patients (e.g., recurrence controls after surgery, controls of high-risk patients, monitoring of therapy responses) can facilitate the assessment of data on the individual basis. In this respect, the application of the approach of "least significant change" or "critical difference" (other name: reference change value) was suggested [114]. This approach was already implemented for the assessment of laboratory results in the eighties [115] and intensively propagated by Fraser [116]. The Bone Marker Standards Working Group of the International Osteoporosis Foundation and the International Federation of Clinical Chemistry and Laboratory Medicine has now recommended this concept of "least significant change" as useful interpretative tool for bone marker data [25]. The term of "least significant change" defines the difference between two serial results that must exceed the merged inherent variability of the two results to be assessed as statistically true change in consequence of a biologic effect. For the calculation of this threshold, the total variation consisting of the analytical and individual variation is multiplied with a factor based on the selected confidence level with a one-sided or two-sided probability testing [116]. For bone markers, the one-sided probability testing with a 90% confidence level has been recently recommended to calculate the critical differences [14,117]. Studies in healthy subjects showed that the conventional bone markers like OC, BAP, PINP, ICTP, NTX, and CTX exhibited critical differences between 15 to 60% [14]; these differences were higher in urine markers than in serum markers [114]. In clinical practice, the electronic reports of the laboratory data should indicate corresponding critical differences to help clinicians interpret serial results in the follow-up [116]. Although the approach of “least significant change” has been in place for the measurement of bone mineral density in radiology for at long time [118], bone markers are still assessed rather rarely by this concept [57,119]. It would be meaningful for the data comparability between different studies to consider this assessment concept as obligatory part in the study design. But also the clinical decision making process would be improved. This way, skeletal complications can be detected earlier and necessary therapeutic changes can be considered in a timelier way. This is particularly the case for high-risk patients who require more intensive monitoring due to suspicious clinical-pathological data. For example, the anti-resorptive therapeutic effect can be reliably checked by means of a drop in bone marker in patients being treated

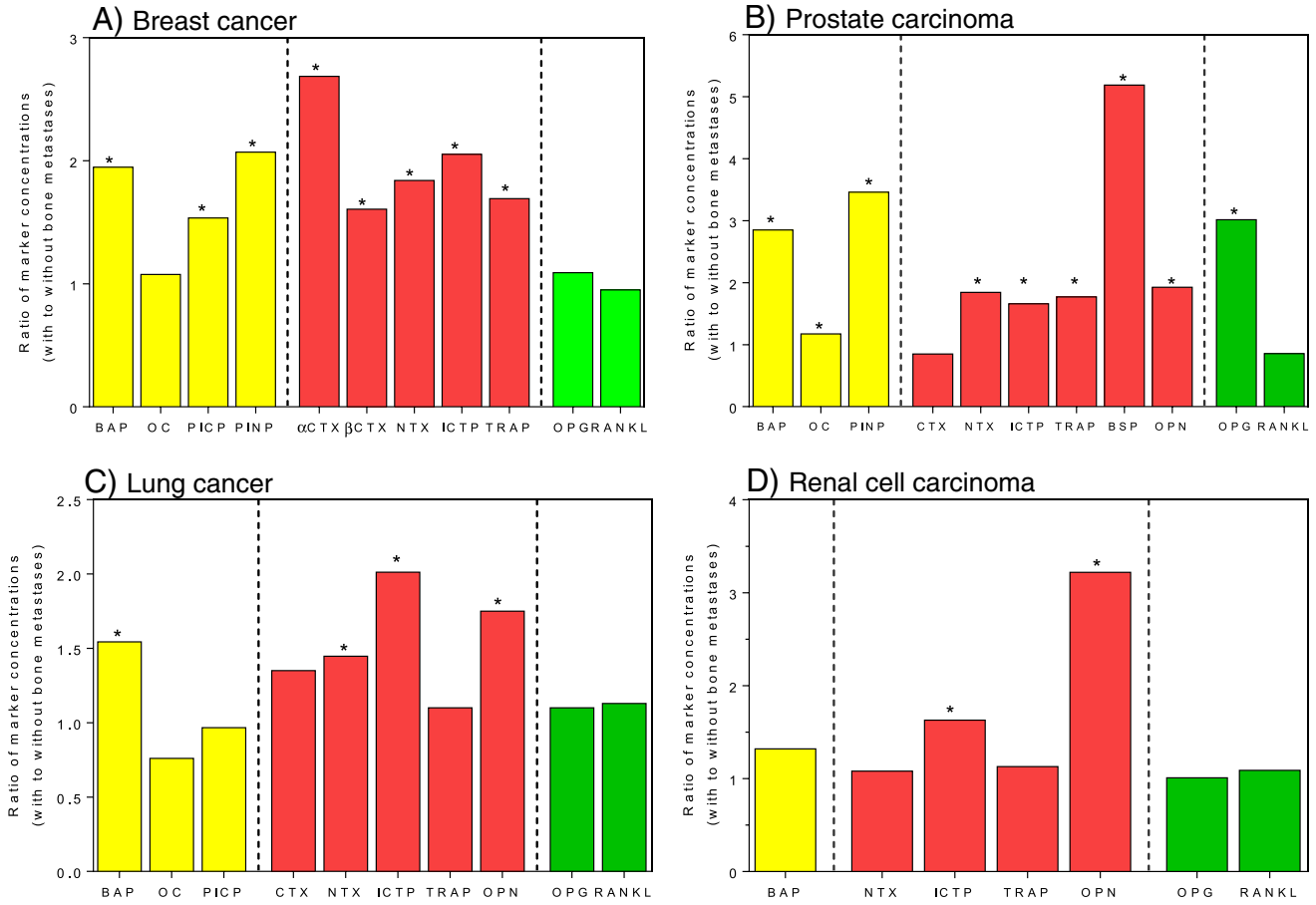

Fig. 1. A–D. Bone markers in serum of cancer patients with bone metastases. The columns represent multiples of bone markers (yellow: bone formation markers; red: bone resorption markers; green: osteoclastogenesis markers) in cancer patients with bone metastases (*, significantly increased) compared to patients without metastases. Values are given as medians or mean values and taken from literature data: (A) Breast cancer [59,89,92–102]; (B) Prostate carcinoma [71,88,103]; (C) Lung cancer [82,89,104–112]; (D) Renal cell carcinoma [90,91]. (Adapted from the figure in the authors chapter in the German textbook "Knochenmetastasen - Pathophysiologie, Diagnose und Therapie" [ISBN 978-3-662-43471-0; Eds.: A. Stenzl, T. Fehm, L.C.Hofbauer, F. Jacob] with kind permission of Springer Science+Bussiness Media, Berlin 2014).

with bisphosphonates to avoid skeletal complications [120]. Bone markers are increasingly used as surrogate parameters in therapeutic studies in order to check the response to a medication [121].

4. Breast cancer

4.1. Bone markers in diagnostics and follow-up examinations

Fig. 1A summarizes the increase in some bone markers in patients with osseous metastases compared to metastasis-free patients from 14 breast carcinoma studies [59,89,92–102]. The analyzed studies described mostly statistically significant increases in the bone marker concentrations with the exception of osteocalcin and the two osteoclastogenesis markers OPG and RANKL. A comparison of the diagnostic sensitivity and specificity of simultaneously measured resorption and formation markers detected diagnostic sensitivities of 42-89% and specificities of 42-95% for the resorption markers (DPD, NTX, PYD, ICTP) and corresponding values of 18-47% and 78 to 97%, respectively, for the formation markers (BAP, PCIP, OC) [92,122]. Particularly the αCTX elimination in the urine is anomalous here. It detected significantly better separation between patients with and without osseous metastases compared to βCTX. However, this data has to date only been published by a workgroup [59]. The BSP that could only be determined with radioimmunoassays in the past also differentiated very well between patients with and without osseous metastases (sensitivity: 89.5%; specificity: 96.7%) [123]. Follow-up examinations detected that values above the upper reference limit of 24 μg/L indicated a high risk of metastasis formation within 2 years after the surgery. However, these promising results have not yet been confirmed in other studies.

The results of various studies are inconsistent even though resorption markers are considered diagnostically more sensitive and formation markers more specific for the detection of osseous metastases in breast carcinoma [122]. For example, the formation marker PINP achieved better diagnostic sensitivity than the resorption marker βCTX. However, the partially contradictory results are not surprising, since other factors can also affect the significance of the results in addition to determination of certain markers. Among these are, (i) the often limited amount and clinical heterogeneity of the patients included in the studies, (ii) the dependency of the markers on the extent of metastasis infiltration [89], (iii) the "unspecific" release of markers from visceral metastases [124] and (iv) insufficiently defined decision limits due to unclear reference intervals [125] as well as (v) the high intra- and inter-individual variability of the bone markers [126]. All of this also caused, amongst other things, the aforementioned wide variation range of diagnostic sensitivity in the various studies. The results of a prospective study on 113 women who were examined every 3 months over a period of 29 months after breast carcinoma removal make this clear [81]. Eleven of these patients developed osseous metastases during the course of the study. However, 93% of all bone marker determinations (amongst others BAP, OC, PICP, NTX, βCTX, PYD, and DPD) detected values that were below a reference threshold determined in a comparison group and thus did not suggest metastasis formation. A similar monitoring study, however, detected elevated concentrations of, e.g. TRAP5b and ICTP, but not βCTX and PINP, in the serum of patients compared to the initial values. Imaging procedures confirmed metastasis formation in these patients afterwards [89]. This underlines how diagnostic reliability of analytical data can be improved on the basis of correctly determined reference thresholds or individual initial values [84]. TRAP5b and ICTP were also described as usable resorption markers by other authors [60,93,94,127]. However, all these bone biomarkers have not become an alternative to imaging diagnostics of osseous metastases in an everyday clinical setting so far [92].

Sparse data exist regarding the new markers DKK-1 and sclerostin. Serum DKK-1 concentrations were found to be significantly increased in women with breast cancer and bone metastases in comparison to women with breast cancer in complete remission or with non-bone metastases and healthy women [128]. However, comparative data on the diagnostic validity were not reported. There were no correlation between DKK-1 values and serum BAP and values in women with bone metastases. DKK-1 was also serially measured in combination with sclerostin, OPG, PINP, and ßCTX in women with breast cancer under the adjuvant treatment with aromatase inhibitors [129]. In the follow-up, an increase of serum sclerostin and modest decrease of DKK-1 were observed after 12 and 24 months of treatment. This different regulation of the two Wnt inhibitors was also connected with an inverse correlation with bone mineral density, namely a positive correlation of sclerostin and a negative one of DKK-1. It might be that sclerostin and DKK-1 could be used for the assessment of bone treatment effects rather than in diagnostics [48].

4.2. Bone markers as prognosticators and in therapeutic studies

The significance of the NTX in the urine and serum of metastasized patients was examined in randomized therapeutic studies. This is the subject of a detailed discussion in the aforementioned consensus publication [27]. In a study with 744 patients, an initial NTX value of > 100 nmol/mmol creatinine in the urine was associated with an early onset of skeletal complications. This was also an indicator of tumor progression as well as a shorter survival time [120]. The prognosis was much worse if this value remained consistent or rose during the course of treatment with zoledronic acid or Pamidronate. The results in the same study with determination of BAP were similar. Comparable prognosis estimations were also possible with determination of NTX in the serum, but different threshold values were used in the two studies [130,131]. Elevated βCTX concentrations in the serum (>710 ng/L) before the start of adjuvant therapy of metastasis-free patients who had received a mastectomy (n = 667) were an indicator of earlier metastasis formation in comparison to the group with lower βCTX concentrations [132]. Zoledronic acid lowers NTX elimination more than Pamidronate. The extent of NTX elimination is considered an indicator of more effective treatment since the risk of skeletal complications in the patients treated with zoledronic acid is lowered parallel to this [120]. Similar predictive results were obtained with PINP measurements in another study that evaluated the adjuvant treatment effect of the antiresorptive agent clodronate on the occurrence of bone metastases after mastectomy [119]. Patients with increased PINP values over 20% at the one year follow-up interval in comparison to the baseline level showed a higher incidence of bone metastases than patients with values below this decision limit during five years of follow-up.

These data show, as already mentioned in the above paragraph, that serial determinations of individual bone markers can result in valuable prognostic and predictive information.

5. Prostate carcinoma

5.1. Bone markers in diagnostics

Fig. 1B shows the relative ratio of relevant bone markers of prostate carcinoma patients who have osseous metastases compared to metastasis-free patients based on data from our own studies [71,88,103]. There is no difference between osteocalcin and RANKL in both groups as in breast carcinoma. All other bone markers have statistically significantly higher concentrations in the metastasized patients. The high PINP, BSP, and OPG values are anomalous in comparison to the other markers. The also significantly exceed the relative bone marker values determined in patients with and without osseous metastasis in breast, lung, and renal cell carcinoma (Fig. 1A–D).

Other workgroups also detected elevated BSP values in metastasized prostate carcinoma patients [133]. However, BSP can be considered a general tumor marker rather than a typical bone biomarker since the concentrations increase in patients with locally confined prostate

carcinoma as well [88,134]. This is apparently also the case for OPN as the other SIBLING protein discussed here [103].

Several workgroups also confirmed elevated OPG levels in the serum of metastasized patients [71,72,135,136]. The OPG concentrations correlate with the extent of osseous metastasis formation. It is presumed that the elevated OPG concentrations are particularly a consequence of increased release from the tumor's microenvironment [137]. Thus, they only indirectly reflect the change in the osseous metabolism. To date there is no diagnostic application of the OPG as a metastasis indicator. However, joint determinations of OPG and RANKL can serve to predict a recurrence after a radical prostatectomy although unchanged RANKL values were found between patients with and without metastases [138]. Moreover, several studies observed that serum RANKL levels were independent of tumor stage, Gleason score, and preoperative PSA [73,88,89,137,138]. In all these studies, the same total sRANKL assay was used, while the general uncertainties of RANKL assays, as briefly stated in Section 2.3.5., should not be overlooked.

Elevated PINP values (mean value: 4.33), as shown in Fig. 1, were also described in four other studies including patients with osseous metastases in comparison to metastasis-free prostate carcinoma patients [89,139–141]. The same goes for BAP as "the" characteristic formation marker. Corresponding to the pattern of the bone markers in Fig. 1, however, not only the formation markers PINP and BAP [89,142,143] are elevated due to osteoblastic metastases typical for prostate carcinoma, but also the classical resorption markers ICTP, TRAP5b, CTX, and NTX [89,140,142–145].

However, the statistically significant changes say little about the diagnostic relevance of the individual bone markers. For example, the study by Garnero et al. [142] detected that all examined bone markers in the serum (BAP, PICP, CTX) and in the urine (αCTX, βCTX) were significantly elevated in patients with osseous metastases compared to those without metastases. The diagnostic sensitivities were only between 53 and 65% here. The authors thus concluded that the named bone markers were not very helpful for metastasis diagnostics.

Skeletal scintigrams were performed in two other studies and different bone markers determined: BAP, ICTP, and TRAP5b [146] or rather BAP, OC, PICP, PINP, NTX, and CTX [76]. BAP correlated best with the extent of osseous changes (bone scan index) and had the greatest diagnostic accuracy (72% sensitivity, 88% specificity). PINP had the highest diagnostic specificity (92%). No clear conclusions were made, however, if and whether bone markers should be used in connection with the skeletal scintigraphy. However, a recent study found unchanged PINP levels in patients who developed bone metastases in comparison with patients with only nodal metastases or PSA relapse [74]. The authors concluded that the PINP would not be a sensitive surrogate marker for early bone metastasis in high-risk prostate cancer patients.

However, there is no information on diagnostic sensitivity and specificity as well as on ROC (receiver-operating characteristics) in many studies. They are minimum criteria so that the diagnostic relevance of the various markers can be compared amongst one another and between different studies. The mean values of the areas under the ROC curve from several studies were 0.81, 0.80, and 0.77 for the presumably most effective markers PINP, BAP, and ICTP [88,140,143,147]. This data shows that these markers can have diagnostic value in interaction with safely defined reference thresholds or in their course.

The new markers sclerostin and DKK-1 deserve a special attention. Gene and protein expression in bone metastatic specimens from prostate cancer patients showed that sclerostin and DKK-1 were not significantly different between osteoblastic and osteolytic metastases [148]. Serum sclerostin values increased in prostate cancer patients with bone metastases by about 40% in comparison to a control group of age-adjusted men [43]. DKK-1 values were not different in 159 men who were diagnosed with cancer or cancer free by prostate biopsy [149]. However, patients who developed bone metastases in the follow-up were characterized by reduced DKK-1 values. These data corrected the first report of the same working group that prostate cancer patients with and without bone metastases had increased values in comparison to healthy men [150]. There are some study results on serum values of sclerostin and DKK-1 in their relationships with age, sex hormones, bone mass, and conventional bone turnover markers in men and prostate cancer patients [44,47,48,151]. However, the current rather unclear state of scientific knowledge does not entitle the use of both analytes in clinical settings, but the inclusion in clinical studies with well-defined objectives seems to be meaningful.

5.2. Bone markers as prognosticators and in therapeutic studies

Significant information was detected in the studies already described for breast carcinoma. These therapeutic studies with zoledronic acid also included metastasized prostate carcinoma patients and affected the markets NTX in the urine and BAP in the serum. Their initial values before the start of the treatment and in the placebo arm as well as in the therapeutic arm were related to the clinical endpoints progression-free interval, skeletal complications, tumor progression, and survival [152]. Patients with high initial NTX values (>100 nmol/mmol creatinine) had a more unfavorable clinical prognosis regarding all of these endpoints than patients with lower values in the placebo arm (203 patients; examination period of 24 months). Follow-up determinations of NTX improved the prognostic significance of the marker. BAP determinations were altogether less significant. In contrast, high BAP concentrations in the serum in the therapeutic arm in patients with a metastasized, castration-resistant prostate carcinoma ($n = 643$) were closely correlated with a shorter survival time. BAP, but not NTX turned out to be an independent factor in a multivariate model [153]. A similar study also confirmed this for patients with a metastasized, androgen-dependent prostate carcinoma [154]. A decrease in the NTX elimination and BAP concentration in the serum during treatment (zoledronic acid; Docetaxel) was equivalent to a longer survival time in this study for patients. Rajpar et al. [155] also detected this prognostic significance of NTX determinations in the urine. The results led to determination of NTX in the urine and BAP in the serum in the follow-up examination of patients with metastasized prostate carcinoma [154]. Saad et al. [156] detected sufficient evidence from the data of the therapeutic studies with zoledronic acid to apply these two biomarkers for therapeutic control with zoledronic acid.

But other bone markers also turned out to be useful indicators: in our own examinations with metastasized patients in who NTX was not detected in the urine, the concentrations of PINP and ICTP were the most significant biomarkers for survival after 15 months of zoledronic acid treatment [157]. The markers βCTX and PINP before and 3 months after the start of treatment were informative regarding the prognosis of survival in a prospective multicenter study of 98 patients with osseous metastases during zoledronic acid treatment; BAP and PINP were prognostic indicators of skeletal complications [158]. In a Japanese study, ICTP determinations detected a more reliable prognosis of the likelihood of survival in comparison to BAP and TRAP5b [143]. Lower initial values of PINP, PICP, and NTX in the serum were also associated with a longer survival time and longer progression-free interval in another drug study with hormone-refractory prostate carcinoma patients [159]. Other authors also confirmed the prognostic relevance of PINP in metastasized prostate carcinoma patients and recommend combining this with determinations of NTX or CTX as well as of the growth factor YKL-40 [160]. Determinations of various bone markers were already used as surrogate parameters of therapeutic efficacy in reflection of the osseous metabolism in phase 1 and 2 drug studies of osseous metastases [161].

Prognostic information from other bone markers relate to the metastasized patients' likelihood of survival by means of BSP determinations [88] and the likelihood of developing a recurrence after a radical prostatectomy using preoperative RANKL and OPG determinations [138]. In a multivariate analysis including conventional clinical-

pathological risk factors, high RANKL concentrations and RANKL/OPG quotients were independent risk factors of recurrence.

6. Lung cancer

6.1. Bone markers in diagnostics

Fig. 1 summarized the ratio of the concentrations of bone markers in the serum of patients with osseous metastases in comparison to metastasis-free patients from 11 studies [82,89,104–112]. The overview is only of an orientating character. Most studies only examined few and different bone markers, for which reason the respective column in the figure contains a maximum of four values. It is anomalous, however, that the relative ratios are lower than in metastasized breast or prostate carcinoma. The mean values of the bone markers of metastasized patients do not exceed 1.5 times the mean values of metastasis-free patients, with the exception of ICTP and OPN. Only ICTP, BAP, NTX, and OPN were indicated as statistically significantly elevated in the original publications, while there is conflicting data on the other markers. Leeming et al. [89] described that αCTX changes reflected the extent of osseous changes as a result of metastases more sensitively than other bone markers (amongst other things BAB, NTX, βCTX, ICTP, and TRAP5b). Patients with and without osseous metastases can also be differentiated with BSP determinations. The diagnostic sensitivity was 77.8%, the specificity 81.1% [162].

Statistically significant differences in the different bone markers between patients with and without metastases or control groups were often described (overview by Mountzios et al. [163]), but sufficient diagnostic sensitivity that meets the clinical requirements can not be expected due to the slight differences. Yao et al. [106] also came to these conclusions. They described statistically significantly higher TRAP5b concentrations in patients with osseous metastases, but emphasized that the low diagnostic sensitivity and specificity did not allow for a robust test. Information on diagnostic sensitivity and specificity is also missing in many studies as already mentioned in the paragraph on prostate carcinoma. Furthermore, the data analyses were also inconsistent, making exact evaluation of a marker more difficult. This is illustrated in an example. Two studies reported comparable diagnostic sensitivities of 66 and 71% for the determination of osseous metastases by ICTP determinations with the same analysis method [104,105]. However, since one decision limit that was taken as a basis was twice as high as the other (4.9 μg/L vs. 9.9 μg/L), the actual clinical validity of the marker can not be evaluated clearly.

There are no convincing recommendations for diagnostics of osseous metastases by bone marker determinations in lung cancer. Tanko et al. [164] ruled out the use of bone markers for this at the current time in a summarizing interpretation of available data, amongst others the data of Ebert et al. [82]. The authors of a recently published, very extensive study of lung cancer patients with (n = 130) and without (n = 135) metastases in which BAP, TRAP5b, and ICTP were determined came to a similar conclusion [165].

6.2. Bone markers as prognosticators and in therapeutic studies

Mountzios et al. [163] compiled the prognostic relevance of bone markers in lung cancer with skeletal metastases in an overview. Particular emphasis was on the therapeutic studies on breast and prostate carcinoma with zoledronic acid treatment already discussed, since they allowed for high statistical safety of the results based on the design (number of patients, multicentric, randomized) [152]. The prognostic statements in the placebo arm of the 238 lung cancer patients with osseous metastases were the same as in prostate carcinoma [152]. Patients with low initial NTX values (<100 nmol/mmol creatinine) had a significantly better prognosis in regards to the clinical endpoints progression-free interval, skeletal complications, tumor progression, and survival than patients with higher values. The greater significance of NTX determinations during treatment and in comparison to BAP determinations was also consistent with the results in prostate carcinoma. An initial NTX value of >100 nmol/mmol creatinine in the urine in the therapeutic arm of the zoledronic acid study meant double the risk of tumor progression, skeletal complications, and a lower likelihood of survival for the patient [120]. A recently published study of 176 lung cancer patients confirmed the prognostic significance of NTX for the serum as well. Pre-therapeutic determinations of NTX with values of >22 nmol/L were associated with a lower likelihood of survival [107]. We can only hope that the results of the NTX determinations in the urine are also transferable to the NTX determinations in the serum. This would certainly make the practical implementation of the test and its acceptance easier.

ICTP and TRAP5b were described as prognostic indicators of tumor progression and of survival in other studies [104,106,108,166].

7. Renal cell carcinoma

Fig. 1 is a summary of our data from various studies on bone markers in renal cell carcinoma [90,91]. It was shown that patients with osseous metastases of renal cell carcinoma did not have significantly high values compared to patients without metastases [90,91]. The concentrations of the bone markers BAP, NTX, TRAP5b, ICTP, OPN, OPG, and RANKL in patients with osseous metastases also did not differ significantly from the values with metastasis formation of the renal cell carcinoma in other organs. Only one other study described significantly elevated PINP values in patients with renal cell carcinoma with metastases (n = 6) in comparison to 24 patients without metastases. The values were lower in another 6 patients with osseous metastases during Sorafenib therapy [167]. The bibliographical data on bone markers in metastasized renal cell carcinoma is poor in comparison to the previously discussed tumors. The use of bone markers for diagnostics of osseous metastases in the everyday clinical practice can currently thus also not be justified scientifically.

The data on the prognostic significance of bone markers in patients with renal cell carcinoma is currently also sparse. Wood and Brown [168] described previously unpublished results that they collected from the previously discussed therapeutic studies in an overview. This resulted in a sub-study with 55 patients with metastasized renal cell carcinoma. The bone markers BAP and βCTX were determined before the start and during zoledronic acid treatment in 29 patients. There was a close correlation between the NTX elimination detected during treatment and tumor progression, a risk of fracture, and the likelihood of survival despite this very low number of patients. βCTX, BAP, and PINP were determined in the serum before treatment and at increments of 3 months in a Spanish therapeutic study (TUGAMO study) on 39 metastasized patients who also received zoledronic acid for 18 months [169]. Patients with elevated pre-therapeutic βCTX levels had a lower likelihood of survival and a higher risk of tumor progression than patients with lower values. Elevated initial BAP values were also associated with an increased risk of skeletal complications. PINP was not associated with the named clinical endpoints in any way.

Various bone markers (BAP, NTX, ICTP, TRAP5b, OPN, OPG, and RANKL) were measured in the serum of metastasized patients with renal cell carcinoma and a multivariate analysis performed in two further studies [90,91]. OPN and OPG turned out to be prognostic indicators of survival. The group of the metastasized patients included patients with osseous metastases as well as with metastases in other organs. It is thus questionable whether the OPN and OPG was solely associated with the osseous metastases, since both markers, as discussed multiple times already, not only occur in the bone.

8. Various tumors

There are few reports on bone marker examinations in osseous metastases of carcinomas other than those already described. This is usually the case in studies that describe changes in the bone markers

as a metastasis effect, but not always take the connection to the primary tumor into consideration.

The Spanish TUGAMO study mentioned in the paragraph on renal cell carcinoma had also included 34 patients with osseous metastases of an urothelial carcinoma [169]. BAP, βCTX, and PINP in this substudy showed that patients with higher BAP and βCTX concentrations at baseline and at three months of follow-up were associated with an increased risk of death (univariate Cox-regression analysis), while increased PINP values predicted the appearance of new skeletal related events at a shorter time interval (Kaplan-Meier analysis). A further study on bladder carcinoma only describes the positive correlation between the amount of the osteopontin concentration in the plasma and the tumor stage [170]. A similar statement was made for ICTP in the serum in hepatobiliary tumors and in pancreatic carcinoma [171]. NTX in urine was also analyzed in patients with metastasized nasopharyngeal carcinomas [172]. This was performed within the scope of a phase II therapeutic study with zoledronic acid as discussed in breast and prostate carcinoma. The treatment with zoledronic acid caused a drop in the initial NTX concentration by 62% after one month already.

We will only mention bone marker determinations in multiple myeloma that is characterized by specific osteolytic bone destructions. Overviews contain specific information on this [173,174].

9. Conclusions and future directions

This review summarizes current aspects of the use of bone turnover markers as indicators of bone metastasis. Much was expected of bone marker determinations to solve diagnostic, prognostic, and therapeutic tasks in patients with osseous metastases. However, this has been only partially accomplished to date despite numerous promising study data as discussed in the previous paragraphs for the corresponding cancers. The results of the numerous studies are often contradictory. There are uncertainties particularly in the diagnostic application of bone markers. Thus, based on the current level of knowledge, there is the conclusion justified that the routine use of these biomarkers cannot be recommended for all the above mentioned clinical settings at this time.

However, this recent rather negative assessment for bone markers as part of routine clinical program does not mean an end for these markers as already unequivocally emphasized by Kleerekoper [175] in 2001. After analysis of bibliographical data it can be presumed that these negative results can partially be explained by insufficient analytics, bad study design, and inappropriate data analysis. The keywords here are: pre-analytics, assay methodology, determination of comparisons, reference ranges, heterogeneity of the study groups, sample-size calculation of study participants, analysis algorithms. We have reviewed all these deficiencies in the previous sections of this article. A critical analysis of these points can help to take the necessary measures to prevent these pitfalls from being continued or repeated. On the other hand, they should also be considered as starting points to intensify efforts for forthcoming challenges of exploiting the true potential of bone markers.

Further progress in the field of bone markers can be expected, roughly spoken, in three directions and challenges: (i) basic research, (ii) translation of new findings into a routine laboratory medicine, and (iii) planning and realisation of clinical trials on the principles of evidence-based medicine to introduce new markers or complement existing approaches. The following comments reflect our opinion on some particular aspects regarding these development steps.

We believe that the guidelines for the development of cancer biomarkers, which were elaborated by the Early Detection Research Network at the U.S. National Cancer Institute, might also be applicable for the development of bone markers. A formal structure of five phases (preclinical exploratory, clinical assay and validation, retrospective longitudinal, prospective screening, cancer control) serves in this guideline as directive for the process of biomarker development [176].

Current basic bone research underlines its important role as immanent source for innovative strategies [41,177,178]. Based on high throughput results in genomics and proteomics the insights into new mechanisms of bone formation and bone resorption could be essentially improved [87]. Differently cooperative interactions between osteoblasts, osteoclasts, and osteocytes and their microenvironment have been discovered [49,50]. These new findings have essentially contributed to a better understanding of bone metabolism as result of a complicated network of numerous dynamic processes. Also potential novel markers and corresponding drug targets for bone diseases have been identified [42,148]. Thus, the clinical usefulness of numerous new markers needs to be validated in future [42,148]. Even already at this early stage of translational research, a close cooperation between clinicians and laboratory scientists is an essential demand to avoid possible pre-analytical pitfalls in collecting and handling of samples. Structured clinical questions considering the core principles of evidence-based laboratory medicine as summarized by Price and Christenson [179] in their standard text book should assist the translation from basic bone research toward biomarker tools for diagnosis, prognosis, and prediction of disease. The above discussed conflicting data of DKK-1 and sclerostin in serum obtained in different studies with patients of the same cancer types underline this aspect and might be a lesson. Due to the insufficient knowledge about the detected fragments in case of sclerostin using different assays [44] or the selection of inappropriate control groups in the DKK-1 studies [53,54] opposite results were achieved. The consensus publications on bone marker analyses in malignant osseous and osteoporosis provide the basis to better accommodate all these aspects in the future [23–25,27,175]. There is the general tendency in laboratory medicine to standardize/harmonize assays on the basis of the traceability of measurements using a reference system in order to obtain a better comparability of results [180–182]. This concept of traceability should also be considered for all new markers and catched-up for the established markers. As shown for other analytes, differences between the measurements with assays from different producers can therefore be diminished [183]. All these efforts should be focused on the development of robust assays. Automated assays should be preferred because of their better imprecision data resulting in a more reliable interpretation of data on the "least significant change" concept, for example.

Prospective multicenter clinical trials are the last step to validate the usefulness of a biomarker for clinical practice. For this, recommendations of accepted guidelines for the assessment of tumor markers and diagnostic accuracy should strictly be considered to avoid faults of study design and in consequence subsequent problems at a later date [184–186]. There is the general rule to compare the index marker that means the marker in validation, with the best marker as "gold standard" up to this point [184]. However, the validation of bone markers is more difficult and needs a more complex approach. Bone resorption and bone formation, especially under treatment conditions, may be affected differently as previously discussed. This situation needs a carefully selected panel of markers for the purpose of comparison and validation. The selection of such a panel should be based on previous pilot results and literature data. The technical feasibility of automated multiplex assays for several bone markers has been demonstrated [187]. A pattern-based assessment has a particular advantage for the prognostic and predictive validation of the respective marker as the marker should be assessed always regarding his significance in the model and not alone [188]. We believe that in addition to the intelligent assessment of data using the “least significant change” approach for single markers (see Section 3.3.), a final decision algorithm based on the combined use of several markers together with imaging and/or clinical data will be possible in the future.

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