

**Relevanz biochemischer Tumormarker für das
Gesamtüberleben von Patienten mit metastasiertem
kastrationsresistentem Prostatakarzinom unter
[Lu177]-PSMA-Therapie**

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Abkürzungsverzeichnis

AP	Alkalische Phosphatase
BAP	knochenspezifische Alkalische Phosphatase
CgA	Chromogranin A
GBq	Gigabecquerel
LDH	Laktatdehydrogenase
mCRPC	metastasiertes kastrationsresistentes Prostatakarzinom
PET/CT	Positronen-Emissions-Tomographie plus Computertomographie
pro-GRP	Pro Gastrin Releasing Peptide
PSA	Prostata-spezifisches Antigen
PSMA	Prostata-spezifisches Membranantigen
PSMA-RLT	PSMA – Radioligandentherapie
RLT	Radioligandentherapie

1. Deutsche Zusammenfassung

1.1 Einleitung

Das Prostatakarzinom ist eine weltweit oft gestellte Diagnose und in Deutschland mit 25,4 % das am häufigsten diagnostizierte Karzinom des Mannes. 2016 lag die Inzidenz der Neuerkrankungen in der Bundesrepublik Deutschland bei ca. 58.800 (Krebs in Deutschland für 2015/2016. 12. Ausgabe. Robert Koch-Institut (Hrsg) und die Gesellschaft der epidemiologischen Krebsregister in Deutschland e.V. (Hrsg). Berlin, 2019). Im Falle einer Metastasierung und eines Fortschreitens der Erkrankung unter Androgendeprivation spricht man vom Stadium des metastasierten kastrationsresistenten Prostatakarzinoms (mCRPC). In diesem Stadium sind meist die leitliniengerechten Therapiemethoden ausgeschöpft und die Patienten überleben ab diesem Zeitpunkt im Durchschnitt nur noch weniger als 20 Monate (Fendler et al., 2017). Daher wird seit einigen Jahren intensiv an weiteren Therapiemöglichkeiten für diese Patienten geforscht.

Da bei Fortschreiten der Erkrankung eine Zunahme der Expression des Prostata-spezifischen Membranantigens (PSMA) beobachtet wurde, bot sich dieses als potentieller Ansatzpunkt für zielgerichtete Therapien im Stadium des mCRPC an (Santoni et al., 2014). Die PSMA-Radioligandentherapie wurde mit dem Hintergrund entwickelt, dass nuklearmedizinische Verfahren schon lange in der Krebstherapie angewandt werden. Gamma- oder Positron-Strahler werden v.a. für diagnostische Zwecke und Beta-, Alpha- oder Auger-Strahler für therapeutische Zwecke genutzt (Jamous et al., 2013). 2015 veröffentlichte die Heidelberg Study Group erste erfolgreiche Ergebnisse zur PSMA-RLT mit dem theragnostischen Marker PSMA 617 und dem Betastrahler Lutetium 177 (Benešová et al., 2015).

Über die verstärkte PSMA-Expression wird versucht, alle das Oberflächenantigen tragenden Tumorzellen zu detektieren. Durch Bindung an den Rezeptor kann dann das radioaktiv markierte Molekül von der Zelle aufgenommen werden und die Tumorzelle von innen bestrahlen (Afshar-Oromieh et al., 2015; Rajasekaran et al., 2003).

Die PSMA-RLT gilt zurzeit noch als letzte Behandlungsoption, konnte aber bereits im Vergleich zu anderen Drittlinientherapien als sicher und effektiv eingestuft werden (Rahbar et al., 2017). Trotzdem ist die aktuelle Indikation für die Anwendung der PSMA-RLT sehr strengen Auflagen unterlegen. Konkret darf sie nur im Rahmen klinischer Studien oder unter strengen Kontrollen bei Patienten, die das Stadium des mCRPC erreicht haben nach Ausschöpfung aller etablierten Standardtherapien angewandt werden (Fendler et al., 2017).

Um die Effektivität der PSMA-RLT daher noch genauer beurteilen zu können, ist es unter anderem wichtig, mögliche Prädiktoren für ein besseres Gesamtüberleben zu evaluieren. Ziel dieser Studie war es, bestimmte biochemische Marker in Korrelation mit dem Gesamtüberleben bei Patienten im Stadium des metastasierten kastrationsresistenten Prostatakarzinoms, welche mit PSMA-Radioligandentherapie behandelt wurden, zu setzen.

Sechs verschiedene biochemische Tumormarker, die alle im Zusammenhang mit Prostatakrebs stehen, wurden in dieser Studie auf ihren prädiktiven Wert untersucht.

Ein wichtiges Enzym, vor allem im Zusammenhang mit Metastasen, ist die Alkalische Phosphatase. Hierbei handelt es sich um ein membrangebundenes Glykoprotein, welches die Hydrolyse von Phosphorsäureestern katalysiert. Es gibt vier Isoenzyme, die in unterschiedlicher Häufigkeit in verschiedenen Geweben vorkommen. Dünndarm-AP, Plazenta-AP, Keimzell-AP und Gewebe-unspezifische AP. Die Gewebe-unspezifische AP unterteilt sich nochmals in Leber/Knochen/Nieren-AP (Sharma et al., 2014). Da der Großteil der im Serum messbaren AP von den Zellen der Leber und des Knochens sezerniert wird, ist der Wert im Zusammenhang mit Erkrankungen dieser Organsysteme klinisch bedeutsam. Vor allem maligne Tumore, welche, wie das Prostatakarzinom, in den Knochen metastasieren, zeigen oftmals AP-Erhöhungen. Es konnte bereits in vielen Studien gezeigt werden, dass ein AP-Wert unter 220U/L mit einem längeren Überleben assoziiert ist (Bräuer et al., 2017; Rahbar et al., 2017; Rahbar et al., 2018).

Als spezifischerer Wert bei Knochenmetastasen gilt die Knochenspezifische Alkalische Phosphatase. Diese wird von den Osteoblasten synthetisiert und spiegelt die knochenbildende Aktivität der Osteoblasten wider (Roudsari und Mahjoub, 2012). Bisher

ist jedoch noch nicht bekannt, inwiefern Veränderungen der AP und BAP nach der Behandlung mit PSMA-RLT eine Aussagekraft bezüglich des Überlebens bei Patienten mit Knochenmetastasen haben könnten.

Weltweit im klinischen Alltag verbreitet und bereits seit 1987 als diagnostischer Marker bei Prostatakrebs eingesetzt ist das Prostataspezifische Antigen. Dieses ist ein Glykoprotein und wird von den Drüsenzellen der Prostata sezerniert (Wang et al., 1979). Trotz relativ niedriger Spezifität und Sensitivität gilt es immer noch als wichtigster Parameter zur Früherkennung sowie als Verlaufsparemeter maligner Erkrankungen der Prostata (Dimakakos et al., 2014). In bisherigen Studien konnte der PSA-Wert außerdem als bedeutsamster Tumormarker im Hinblick auf das Therapieansprechen und das Post-PSMA-RLT-Überleben definiert werden (Ahmadzadehfar et al., 2017).

Als weiterer biochemischer Marker wurde die Laktatdehydrogenase beobachtet. Diese ist ein ubiquitär vorkommendes zytoplasmatisches Enzym und dadurch ein guter Parameter für vermehrten Zellumsatz. Es ist eines der Schlüsselenzyme der anaeroben Glykolyse, welches die Umwandlung von Pyruvat zu Laktat katalysiert und umgekehrt (Farhana und Lappin, 2020). Nach dem Warburg-Effekt wird bei Krebszellen auch unter aeroben Bedingungen die Glykolyse mit Ausscheidung von Laktat betrieben. Dies ist sonst nur in anaeroben Stoffwechsellagen der Fall. Die abnormen Krebszellen profitieren insofern davon, dass sie die Bildung von oxidativem Stress durch die Elektronentransportkette umgehen können. Außerdem können die Tumorzellen so Zugang zu den Stoffwechselzwischenprodukten des Zitratzyklus erhalten, um Lipide und Nukleinsäuren für eine schnellere Zellproliferation zu bilden (Liberti und Locasale, 2016). Bei Zelluntergang wird LDH aufgrund seines ubiquitären Vorkommens freigesetzt und ist dann im Serum messbar (Farhana und Lappin, 2020).

In bisherigen Studien wurde noch nicht die Rolle der neuroendokrinen Tumormarker Chromogranin A und pro-GRP bei Patienten mit mCRPC und PSMA-RLT untersucht. Chromogranin A gehört zur Familie der Granine, einer sekretorischen Proteinfamilie und kommt in endokrinen, neuroendokrinen und Immunzellen vor. Erhöhte Werte finden sich bei Patienten mit neuroendokrinen Tumoren, Herzinsuffizienz, Niereninsuffizienz, Bluthochdruck, Rheumatoider Arthritis und entzündlichen Darmerkrankungen (Hendy et al., 1995). In einer Literaturrecherche wurde diskutiert, ob erhöhte CgA-Werte signifikant

mit einem schlechteren Gesamtüberleben korrelieren (Guo et al., 2019). Es fehlen jedoch noch Studien mit größerer Stichprobe, weshalb in unserer Studie dieser Wert ebenfalls eingeschlossen wurde. Das Pro Gastrin Releasing Peptide ist ein Vorläuferhormon, welches durch Amidierung zu dem aktiven Endprodukt Gastrin Releasing Peptide wird. Es steht im Verdacht als Wachstumsfaktor für verschiedene Krebsarten zu dienen, unter anderem für Prostata Krebs (Dumesny et al., 2006).

Ziel unserer Studie war es herauszufinden, ob die biochemischen Marker AP, BAP, PSA, LDH, CgA und pro-GRP das Überleben der mit PSMA-RLT behandelten Patienten vorhersagen können. Des Weiteren wurden die Veränderungen der Tumormarker während und nach den einzelnen PSMA-RLT-Zyklen beurteilt und in Korrelation mit dem Gesamtüberleben gesetzt.

1.2 Material und Methoden

Die Studie umfasst 137 Patienten, welche im Zeitraum von Januar 2015 bis November 2017 mit PSMA-RLT behandelt worden sind. Alle Patienten sind bis zu ihrem Tod regelmäßig untersucht worden.

Voraussetzung zum Studieneinschluss war der Nachweis PSMA-positiver Metastasen. Diese wurden mittels [68Ga]Ga-PSMA-11-PET/CT vor Therapiebeginn festgestellt. Das Behandlungsprotokoll dieser Studie erfüllt alle ethischen Standards und alle Patienten haben Einverständnis zur Durchführung der Therapie und Nutzung ihrer Daten für die retrospektive Analyse gegeben.

Die Tumormarker wurden vor jedem PSMA-RLT-Zyklus und bei den Nachuntersuchungen durch Blutentnahmen bestimmt. Das Intervall zwischen den Zyklen betrug 6-8 Wochen, das der Nachuntersuchungen 2-3 Monate. Der PSA-Wert wurde zusätzlich für einen Zeitraum von 8 Wochen nach Behandlung zweiwöchentlich sowie bei jeder Nachuntersuchung bestimmt.

Zur Berechnung des Gesamtüberlebens und für die statistischen Analysen wurden vorerst die Patientendaten in einer Datenbank zusammengefasst. Das Gesamtüberleben wurde ab Beginn der PSMA-RLT berechnet. Für die Überlebenszeitdaten wurde ein Median mit

einem Konfidenzintervall von 95% gebildet und mittels Kaplan-Meier-Kurve graphisch dargestellt. Für die deskriptive Statistik, Frequenzanalyse und statistische Vergleiche wurde das Softwareprogramm SPSS genutzt. Die Korrelation zwischen den einzelnen Tumormarkern und dem Gesamtüberleben wurde mit Hilfe des Log-Rank-Tests, multivariable Analysen wurden mittels Cox-Regression ermittelt. Das Signifikanzniveau wurde auf $p < .05$ gesetzt.

Zur Unterteilung der Patienten in Untergruppen wurden Grenzwerte für die verschiedenen Tumormarker festgelegt. Diese waren in den meisten Fällen identisch mit den oberen Normwerten. 120 U/L für AP, 248U/L für LDH, 63pg/ml für pro-GRP und 100ng/ml für CgA. Der PSA-Grenzwert wurde durch das 1. Quartil der Studienkohorte festgelegt und war demnach bei 47 ng/ml. Um in der Analyse von einer Veränderung der Tumorwerte sprechen zu können, musste ein Anstieg oder Abfall des Ausgangswertes um mindestens 25% vorliegen. Veränderungen unter 25% galten als stabile Werte. Einzige Ausnahme bildete die Veränderung des PSA-Wertes. Diese richtete sich nach den Empfehlungen der Prostate Cancer Working Group 3 (PCWG3), die einen Abfall des PSA-Wertes um $\geq 50\%$ als teilweises Ansprechen und einen Anstieg von $\geq 25\%$ als Krankheitsprogress definiert. Ein Anstieg unter 25% oder ein Abfall unter 50% galten demnach als stabile Erkrankung (Scher et al., 2016).

Unsere Untersuchungen beziehen sich auf die Veränderungen der Tumormarker nach dem ersten Zyklus der PSMA-RLT sowie Veränderungsmuster während der Behandlung und bei den Nachuntersuchungen. Zur Auswertung der Veränderungsmuster werden die folgenden vier Beschreibungen angewandt: stetig abfallend, stabil, stetig ansteigend und schwankend. Schwankend wäre beispielsweise erst abfallend/stabil und dann ansteigend oder umgekehrt.

1.3 Ergebnisse

Zum Zeitpunkt des Therapiebeginns hatten alle Patienten einen Eastern Cooperative Group Performance Status (ECOG) von 3 oder weniger und ein Serumkreatinin unter 2,0mg/dl. Außerdem wiesen alle Patienten Metastasen auf, 98% Knochenmetastasen und 75% Lymphknotenmetastasen. Die Kohorte hatte ein medianes Alter von 71 Jahren sowie

einen medianen Gleason-Score von 8. Bei Studienbeginn hatten bereits 97% der Patienten eine antihormonelle Therapie erhalten, 52% bekamen weiterhin eine Zweitlinien-Hormontherapie mit Präparaten wie Abiraterone oder Enzalutamide. 72% hatten zuvor eine zytotoxische Chemotherapie verabreicht bekommen, von denen 35% dieser Patienten zusätzlich noch mit einer Zweitlinienchemotherapie behandelt worden sind. 62% nahmen Bisphosphonate oder RANKL ein und 34% erhielten eine Therapie mit Ra-223 (vgl. Table 1 der beigefügten Originalpublikation). Den Patienten wurde eine Gesamtzahl von 487 Zyklen verabreicht, im Median erhielt jeder Patient 3 Zyklen mit einer medianen verabreichten Aktivität von 6,2 GBq pro Zyklus. Das Intervall zwischen den Zyklen betrug im Median 7,5 Wochen. Die endgültige Überlebensanalyse wurde nach dem Versterben der Patienten durchgeführt, was im Median 50 Wochen nach Therapie war (2-177 Wochen).

Das Gesamtüberleben korrelierte sowohl positiv mit der Anzahl der durchgeführten Zyklen als auch mit der Höhe der kumulativen Aktivität. Patienten, welche nach der PSMA-RLT noch eine andere Behandlung - meist Chemotherapie - erhalten hatten, konnten keinen signifikanten Überlebensvorteil aufweisen.

Normale Ausgangswerte für AP, LDH und PSA bei Behandlungsbeginn haben signifikant mit einem längerem Gesamtüberleben korreliert, während die Ausgangswerte der neuroendokrinen Marker CgA und pro-GRP keinen prädiktiven Charakter hatten (vgl. Figure 1 und 2 der beigefügten Originalpublikation).

Die Reaktionen der Tumormarker auf den ersten PSMA-RLT-Zyklus haben in den meisten Fällen mit dem Überleben korreliert. Ein Rückgang zuvor erhöhter Werte nach dem 1. Zyklus konnte bei AP, LDH, PSA und pro-GRP ein signifikant längeres Gesamtüberleben zeigen.

133 der 137 Patienten hatten Knochenmetastasen und wurden hinsichtlich der Veränderungen der AP und BAP-Werte untersucht. Um den AP-Wert in Relation mit dem BAP-Wert zu setzen und somit besser interpretieren zu können, wurden die Patienten nach Anzahl der Metastasen in 4 Gruppen unterteilt: Unter 6 Metastasen, 6-20, mehr als 20 und disseminierter Befall. Patienten mit stabilen oder zurückgegangenen AP-Werten während der Therapie haben einen signifikanten Überlebensvorteil gegenüber Patienten

mit ansteigenden Werten gezeigt. Bei Patienten, die zumindest stabile BAP-Werte hatten, konnte ebenfalls ein besseres Gesamtüberleben als bei Patienten mit ansteigenden Werten verzeichnet werden (vgl. Figure 5 der beigefügten Originalpublikation). Jedoch konnte in keiner der Untergruppen eine überlegene Korrelation von BAP mit dem Gesamtüberleben gegenüber AP nachgewiesen werden.

Die beiden neuroendokrinen Tumormarker besaßen vor Therapiebeginn keinen prädiktiven Wert im Hinblick auf das Gesamtüberleben und für CgA ergab sich auch unter Therapie keine signifikante Aussagekraft. Ein pro-GRP-Abfall unter Therapie nach zuvor erhöhten Werten zeigte jedoch ein signifikant längeres Gesamtüberleben als bei Patienten ohne pro-GRP-Abfall (vgl. Figure 4 der beigefügten Originalpublikation).

Zusammengefasst lässt sich sagen, dass diejenigen Patienten, welche abfallende, stabile oder schwankende Werte im Therapieverlauf zeigten, ein signifikant längeres Gesamtüberleben im Vergleich zu Patienten mit stetig ansteigenden Werten hatten.

1.4 Diskussion

Da die PSMA-RLT zurzeit noch als letzte Behandlungsoption für Patienten mit Prostatakrebs gilt, haben alle Patienten zum Zeitpunkt des Behandlungsbeginns bereits das Stadium des metastasierten CRPC erreicht und zeigen in den meisten Fällen Symptome (Ahmadzadehfar et al., 2018; Fendler et al., 2016).

In diesem Stadium präsentiert sich jedoch eine starke Heterogenität bezüglich der Überlebenszeit von wenigen Wochen bis zu einigen Jahren. Daher ist eine genaue Einschätzung dieses Gesamtüberlebens für die Frage nach der Sinnhaftigkeit einer weiteren Behandlung notwendig. So könnten der Verlust wertvoller Lebenszeit und unnötige Nebeneffekte in einigen Fällen verhindert werden (Verhagen, 2009).

Wir konnten Ergebnisse bezüglich des Vorhersagewertes von Tumormarkern auf das Gesamtüberleben festhalten und somit einen Teil dazu beitragen, eine bessere Kenntnis über Patienten im Stadium des mCRPC zu erlangen. Diese könnte zukünftig bei der Planung der Behandlungsoptionen sinnvoll sein.

Trotz Uneinigkeiten in der Literatur, ob die PSA-Konzentration ein verlässlicher Wert ist, um das CRPC zu beobachten, bildet der Wert aufgrund des Mangels an präzisen alternativen Tests die Grundlage für wichtige therapeutische Entscheidungen (Collette et al., 2006; Scher et al., 2009; Smaletz et al., 2002; Verhagen, 2009).

In unserer Studie wurde eine signifikante Korrelation zwischen PSA-Veränderungen und dem Überleben der Patienten im Anschluss an die PSMA-RLT nachgewiesen. Da die Entscheidung über die Fortsetzung der PSMA-RLT jedoch anhand des PSA-Wertes getroffen wurde, ist die Korrelation möglicherweise verzerrt. Dementsprechend korrelierte auch die kumulativ verabreichte Aktivität der PSMA-RLT, wie auch Rahbar et al. (2018) in einer vergleichbaren Studie gezeigt haben, positiv mit dem Überleben.

Ein bei Therapiebeginn erhöhter AP-Wert ist mit einem kurzen progressionsfreien Überleben und einem schlechten Gesamtüberleben bei Patienten mit Prostatakrebs assoziiert. Dies konnte eine große Metaanalyse zeigen, weshalb sich die Bestimmung von AP als kostengünstig, verlässlich und prognostisch wertvoll bei Patienten mit Prostatakrebs erwiesen hat (Li et al., 2018). Die AP wurde außerdem bereits als unabhängiger Marker für Patienten, welche PSMA-RLT erhalten haben, getestet (Barber et al., 2019; Bräuer et al., 2017; Rahbar et al., 2018; Rahbar et al., 2017). In der aktuellen Studie zeigten Patienten mit normalen AP Werten ein besseres Gesamtüberleben als Patienten mit abnorm veränderten Werten. Patienten mit bei Therapiebeginn erhöhten AP-Werten könnten im Falle eines Rückgangs oder einer Stabilisierung des Wertes ein längeres Gesamtüberleben haben.

Wie bereits in der Einleitung erläutert ist der LDH-Wert aufgrund der bei malignen Tumoren vermehrt anfallenden anaeroben Glykolyse ein hilfreicher prognostischer Marker für metastasierte Karzinome (Petrelli et al., 2015). Einige Studien haben zudem den prädiktiven Wert für LDH bei Patienten mit CRPC unter Chemotherapie bestätigt (Belderbos et al., 2017; Cotogno et al., 2018; Halabi et al., 2014; Petrelli et al., 2015; Scher et al., 2009). Für unsere Studiengruppe, in der 137 Patienten bis zum Tod nachverfolgt wurden, ist es uns erstmals gelungen, eine Korrelation zwischen erhöhtem LDH zu Therapiebeginn und kürzerem Gesamtüberleben festzustellen. Ein signifikanter Unterschied konnte außerdem zwischen den Patienten mit initial erhöhten LDH-Werten,

welche dann stabile/sinkende Werte erreichen konnten und Patienten mit weiterhin steigenden LDH-Werten unter Therapie beobachtet werden.

Mehr als zwei Drittel der Patienten mit Prostatakrebs entwickeln im Laufe ihrer Erkrankung Knochenmetastasen (Croucher et al., 2015). Dadurch entstehende Probleme wie häufigere schwerwiegende Ereignisse des Skelettsystems, Störungen der Hämatopoese und des Calciumhaushaltes und Schmerzen bedingen eine insgesamt erniedrigte Lebensqualität und ein kürzeres Gesamtüberleben (Carlin und Andriole, 2000; Coleman, 2006; Oefelein et al., 2002; Pinski und Dorff, 2005).

Die Ergebnisse einer Studie von Smith et al. (2007) haben gezeigt, dass erhöhte BAP-Serumkonzentrationen bei Männern mit hormonresistentem Prostatakrebs und Knochenmetastasen mit einem größeren Risiko ungünstiger skelettaler Ereignisse verbunden sind.

Es gibt unterschiedliche Studien, in denen der BAP-Wert bei Patienten mit mCRPC unter verschiedenen Therapien untersucht worden ist, jedoch noch keine veröffentlichten Daten, welche die Relevanz der BAP-Konzentration im Zusammenhang mit der PSMA-RLT erwähnen (Petrioli et al., 2004; Som et al., 2012). Daher wurde dieser Wert in unserer Kohorte genauer untersucht. Es konnte ein signifikant längeres Gesamtüberleben bei den Patienten gezeigt werden, die zumindest stabile BAP-Werte nach PSMA-RLT erreicht hatten, gegenüber denen mit angestiegenen Werten. Auch Patienten mit Knochenmetastasen und stabilen oder gesunkenen AP-Werten nach Therapie hatten ein signifikant längeres Gesamtüberleben. Dennoch blieb eine überlegene Korrelation des BAP mit dem Gesamtüberleben im Vergleich zum AP in unserer Kohorte aus. Um die Sinnhaftigkeit der routinemäßigen Anwendung der BAP-Bestimmung bei Patienten mit Knochenmetastasen und CRPC abschätzen zu können, bedarf es daher weiterer Analysen.

Das Vorkommen neuroendokrin differenzierter Prostatazellen nimmt mit Fortschreiten der Erkrankung zu. Diese Zellen exprimieren Peptide und Wachstumsfaktoren, welche das Tumorwachstum und die Apoptoseinhibition aktivieren (Abrahamsson, 1999; Yashi et al., 2003). Es wird angenommen, dass eine neuroendokrine Differenzierung durch den Mangel an Androgenrezeptoren mit einer Hormonresistenz assoziiert ist und mit einem

signifikant schlechteren Überleben korreliert (Berruti et al., 2000; Komiya et al., 2009). Im Stadium des metastasierten kastrationsresistenten Prostatakarzinoms gibt es meist keine repräsentativen bioptischen Nachweise mehr, da diese in der Regel bei Diagnosestellung und damit vor der antihormonellen Therapie durchgeführt werden (Verhagen, 2009). In diesem Stadium gibt es allerdings einige Patienten, welche keine PSA-Erhöhung zeigen, weshalb weitere Tumormarker hinsichtlich ihres prognostischen Wertes untersucht worden sind (Deftos et al., 1996). Es stellt sich die Frage, ob neuroendokrine Tumormarker in diesem Stadium genauere Informationen über die neuroendokrine Differenzierung und die Prognose bereitstellen können.

Unsere Studie ist die erste, welche die Relevanz der neuroendokrinen Tumormarker bei Patienten, welche eine PSMA-RLT erhalten haben, untersucht. Sowohl für CgA als auch für pro-GRP konnte bei zuvor erhöhten Werten und stabilen oder rückläufigen Werten im Laufe der Therapie ein längeres Überleben als bei Patienten mit stetig steigenden Werten gezeigt werden. Dieses war jedoch nur für pro-GRP statistisch signifikant. Ein Grund für die stärkere Korrelation des pro-GRP-Wertes könnte das gehäufte Vorkommen erhöhter pro-GRP Werte in diesem bereits sehr aggressiven Tumorstadium sein (Yashi et al., 2002, 2003).

PSA-Veränderungen und deren prognostischer Wert nach verschiedenen Behandlungen sind bereits in einigen Studien genauer untersucht worden (Cavanaugh et al., 2004; Semjonow und Schmid, 2002). Die Korrelation zwischen dem Gesamtüberleben und den Veränderungen der Serumkonzentrationen der biochemischen Marker AP, BAP, LDH und den beiden neuroendokrinen Tumormarkern CgA und pro-GRP nach PSMA-RLT über einen längeren Zeitraum wurde jedoch bisher noch nicht veröffentlicht. Unsere Ergebnisse haben gezeigt, dass die Veränderungsmuster der Tumormarker wichtige Prädiktoren für das Gesamtüberleben bei mit PSMA-RLT behandelten Patienten sind.

In unserer Kohorte haben vor allem die Tumormarker PSA, AP, BAP und LDH eine signifikante Korrelation mit dem Überleben gezeigt. Patienten mit stetig abfallenden oder stabilen Werten hatten ein signifikant längeres Gesamtüberleben als Patienten mit stetig ansteigenden Werten. Die Gruppe der Patienten mit schwankenden Werten hat ebenfalls ein längeres Gesamtüberleben gezeigt, was jedoch durch die kleine Patientenzahl und Heterogenität weniger aussagekräftig ist.

Mögliche Fehlerquellen sind eine eventuelle Verzerrung durch einen teilweisen Mangel an Standardisierung. Außerdem könnte die Korrelation zwischen dem PSA-Wert und dem Überleben in unserer Studie durch die vom PSA-Wert abhängige Entscheidung über den Therapieverlauf beeinflusst worden sein. Vorteile dieser Studie sind die hohe Anzahl der Patienten und die lange Nachuntersuchungszeit, da alle Patienten bis zu ihrem Tod von uns beobachtet wurden. Diese Daten können also für zukünftige prospektive Studien über das Gesamtüberleben unter PSMA-RLT genutzt werden.

1.5 Zusammenfassung

Die Studie hat herausgefunden, dass neben dem PSA-Wert als bereits prognostisch etabliertem Tumormarker auch weitere Tumormarker einen prädiktiven Stellenwert für Patienten mit mCRPC, welche mit PSMA-RLT behandelt werden, besitzen. Stabile und/oder erniedrigte Werte für AP, LDH, PSA, pro-GRP und BAP korrelierten sowohl in der univariaten als auch in der multivariaten Analyse signifikant mit einem längeren Gesamtüberleben. Diese Ergebnisse könnten zukünftig bei der Planung der Behandlungsoptionen hilfreich sein.

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ORIGINAL ARTICLE

The value of tumor markers in men with metastatic prostate cancer undergoing [¹⁷⁷Lu]Lu-PSMA therapy

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Abstract

Background: Currently, prostate-specific membrane antigen-radioligand therapy (PSMA-RLT) is considered a last-line treatment option in advanced castration-resistant prostate cancer. Despite these patients' poor prognosis, accurate estimation of their overall survival (OS) is essential to determine whether benefits exist from the treatment and whether the loss of valuable time and unnecessary side effects can be avoided. The aim of the present study is to evaluate whether various biochemical markers can predict OS in men undergoing PSMA-RLT and whether the changes assessed after PSMA-RLT correlate with the OS.

Methods: The tested tumor markers in this retrospective analysis were alkaline phosphatase (ALP), bone-specific alkaline phosphatase (BAP), prostate-specific antigen (PSA), lactate dehydrogenase (LDH), chromogranin A, and pro-gastrin-releasing peptide (pro-GRP). For the evaluation, we performed blood tests before each PSMA-RLT cycle and during follow-up visits (which were 2-3 months apart). All patients were followed up until their deaths. To test the correlations between the tumor markers and survival, we conducted the logrank tests and the multivariate Cox proportional-hazards regression model. The significance level was set at $P < .05$.

Results: The study included 137 patients who received a total of 487 PSMA-RLT cycles between January 2015 and November 2017. Of the tested biochemical tumor markers, baseline ALP (120 U/L cut-off), LDH (248 U/L cut-off), and PSA (first quartile cut-off) correlated significantly with survival post-PSMA-RLT ($P < .001$ for ALP and LDH, and $P = .007$ for PSA). Stable and/or decreased values in most of the initially abnormal parameters were associated with significantly better OS; these parameters were ALP ($P = .009$), LDH ($P = .005$), PSA ($P < .001$), and pro-GRP ($P = .013$). The BAP and ALP responses also correlated significantly with survival in patients with bone metastases ($P = .002$ and $P < .001$, respectively). Furthermore, there was a strong correlation of the kinetic patterns of PSA, ALP, BAP, and LDH with the survival, showing that patients with steadily increasing markers had the shortest OS.

Conclusion: Along with the established tumor marker PSA, ALP, LDH, BAP, and pro-GRP were correlated with the OS post-PSMA-RLT in the univariate and multivariate analyses.

KEYWORDS

prostate cancer, PSMA, radioligand therapy, tumor markers

1 | INTRODUCTION

Patients with prostate cancer (PC) exhibit a high expression of the prostate-specific membrane antigen (PSMA), especially at the androgen-independent stage and/or in case of progressive disease (PD), and when the disease has spread metastatically.¹ This makes PSMA a potential target for the treatment of patients with castration-resistant prostate cancer (CRPC).²⁻⁶ PSMA-radioligand therapy (PSMA-RLT) is a novel compound that comprises the theranostic agent PSMA-617 and β -emitter lutetium-177, which bind specifically to the PSMA and, following internalization, irradiate the tumor tissue from inside.⁷⁻¹⁰

The first preclinical and clinical experiences using PSMA-617 were published by the Heidelberg study group^{8,11} in 2015, and since then, PSMA-RLT has quickly gained worldwide attention as a treatment for men with CRPC.¹²⁻¹⁵ However, PSMA-RLT is still considered a last-line treatment option, as data from prospective randomized phase III trials are lacking.^{16,17} According to recent studies, PSMA-RLT seems to be safe and effective, even for patients with advanced PC or multiple PC relapses following several therapies.^{15,18-27} In the literature, the typical length of time that patients live posttreatment ranges between 7.5 and 15 months.^{18,22,25,28,29}

Various biochemical markers have been assessed to determine the predictive value of responses and overall survival (OS) following PSMA-RLT.^{20,24,25,30-32} We previously reported that the most important predictive tumor marker for response and survival post-PSMA-RLT is prostate-specific antigen (PSA). On the contrary, baseline alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) did not correlate with the PSA response and were not predictive for the OS in the previous multivariate analysis.^{31,32} However, in several studies, ALP less than 220 U/L has been associated with better survival.^{22,24,25} In previous studies, we did not address the role that neuroendocrine tumor markers, such as chromogranin A (CgA) and pro-gastrin-releasing peptide (pro-GRP), play in men with CRPC who undergo PSMA-RLT. It also remains unclear whether changes in ALP and bone-specific alkaline phosphatase (BAP) posttreatment are predictive for the survival of patients with bone metastases.

The present study, therefore, was aimed to evaluate whether biochemical markers ALP, BAP, PSA, CgA, and pro-GRP can predict survival in men who undergo PSMA-RLT. In addition, we assessed the changes in different markers after the patients' PSMA-RLT cycles and tested the correlation of these with the patients' OS. We included a large number of patients in the analysis, all of whom were followed up until their deaths.

2 | MATERIALS AND METHODS

2.1 | Patients and treatment

Evaluated in the analysis were patients with histologically confirmed metastatic CRPC who had undergone PSMA-RLT. Prior [⁶⁸Ga]Ga-PSMA-11-PET/CT was performed to confirm the patients' PSMA-positive metastases. All patients gave their informed consent before their treatment and gave their permission for their data to be retrospectively analyzed. The treatment protocol of this study occurred in accordance with the clause for compassionate use of the German Medicinal Products Act (§13.2b) and met the requirements of the local ethics committee, the declaration of Helsinki, and comparable ethical standards. To retrieve the data for this analysis, we used clinical records. Each patient's characteristics, including their baseline tumor markers, prior therapies, and tumor spread, are summarized in Table 1. Patients who were receiving proton pump inhibitors were excluded from the CgA analysis.

The radiolabeling, administration, and treatment procedures in this study were conducted using previously described methods.^{15,19-21,26,27,31} The median administered activity per cycle was 6.2 GBq (total range of 3.2-7.5 GBq). All the patients included in this study were followed up until their deaths.

2.2 | Laboratory procedures

In addition to established tumor marker PSA, we also assessed LDH, bone-specific marker BAP in correlation to ALP, and neuroendocrine tumor markers CgA and pro-GRP. For the detection of these laboratory parameters, different methods were applied: a photometric assay using a ultraviolet-visible spectrophotometer was used for the LDH and ALP measurements, BAP was measured spectrophotometrically, CgA was measured using an enzyme-linked immunosorbent assay, and pro-GRP and PSA were each measured using an electrochemiluminescence immunoassay.

To evaluate the different tumor markers, we performed blood tests before each PSMA-RLT cycle (the intervals between the cycles were 6-8 weeks in length) and at the follow-up visits (which occurred every 2-3 months). Furthermore, the tests for the PSA changes were repeated every 2 weeks for a period of 8 weeks posttreatment and at each follow-up visit.

2.3 | Survival calculations and statistical analyses

The data from the clinical records were summarized in a database. The OS was calculated from the initiation of the PSMA-RLT. The

TABLE 1 Patients' characteristics

Characteristic	N
Age (Q1-Q3)	
Median	71 (64-76)
ECOG (%)	
0	61 (45)
1	50 (37)
2	25 (18)
3	1 (<1)
Gleason score (Q1-Q3)	
Median	8 (7-9)
Baseline tumor markers: median (Q1-Q3)	
PSA (ng/mL)	208 (47-693)
ALP (U/L)	160 (80-321)
BAP (µg/L)	46 (17-101)
LDH (U/L)	280 (210-420)
CgA (ng/mL)	123 (77-221)
pro-GRP (pg/mL)	73 (56-111)
Extent of the disease (%)	
Local tumor/recurrence	49 (36)
Bone metastases	133 (97)
<6 metastases	16 (12)
6-20 metastases	30 (22)
>20 metastases	52 (39)
diffuse metastases/superscan	36 (27)
Lymph node metastases	102 (75)
Liver metastases	24 (18)
Other metastases	28 (20)
Prior and ongoing therapies (%)	
Antihormonal therapy	Hx of: 1 (<1), ongoing: 133 (97)
Abiraterone	Hx of: 64 (47), ongoing: 23 (17)
Enzalutamide	Hx of: 45 (32), ongoing: 48 (35)
Bisphosphonate or RANKL inhibitor	Hx of: 14 (10), ongoing: 85 (62)
Chemotherapy	Hx of: 97 (71)
Ra-223	Hx of: 47 (34)

Abbreviations: ALP, alkaline phosphatase; BAP, bone-specific alkaline phosphatase; CgA, chromogranin A; ECOG, Eastern Cooperative Oncology Group; Hx, history; LDH, lactate dehydrogenase; N, number of patients; pro-GRP, pro-gastrin-releasing peptide; PSA, prostate-specific antigen; Q1-Q3, range between the first and third quartiles, RANKL, receptor activator of nuclear factor kappa B ligand.

results were reported as median with 95% confidence intervals (95% CIs) and visualized via Kaplan-Meier curves. For the descriptive statistics, frequency analyses, and statistical comparisons, we used SPSS software (IBM SPSS Statistics 25.0, New York). To determine the correlations between the tumor markers and survival, we conducted logrank tests for the univariate analysis and the Cox proportional-hazards regression model with a stepwise variable addition for the multivariate analysis. The significance level was set at $P < .05$.

To separate the patients into subgroups, we defined cut-offs for the different tumor markers. For most of the markers, we used the upper normal value: 120 U/L for ALP, 248 U/L for LDH, 63 pg/mL for pro-GRP, and 100 ng/mL for CgA. The cut-off for PSA was set at the first quartile of the studied cohort, which was 47 ng/mL.

In the analysis, a tumor marker decrease or increase was defined as a change of at least 25% of the baseline value. Changes less than 25% indicated that the disease was stable. An exception to this rule,

however, was the PSA values, which were classified according to the recommendations of the Prostate Cancer Working Group 3 (PCWG3) as follows: partial response for a PSA decrease $\geq 50\%$ and PD for a PSA increase $\geq 25\%$. A PSA increase less than 25% or decrease less than 50% indicated that the disease was stable (SD).³³

We evaluated changes in the tumor markers after the first cycle of PSMA-RLT as well as kinetic patterns during the treatment and follow-up visits. The kinetics of the different tumor markers were divided as follows: steadily declining, stable, steadily increasing, and mixed, such as first decreasing/stable, then increasing or first increasing, then decreasing/stable values.

3 | RESULTS

3.1 | Patients and treatment

This study included 137 patients who underwent PSMA-RLT during the period between January 2015 and November 2017. All of the patients had an Eastern Cooperative Group performance status of 3 or less, and serum creatinine less than 2.0 mg/dL. Additionally, all of the patients' cases were metastatic; 98% of them had bone metastases and 75% had lymph node metastases. Ninety-seven percent had undergone concomitant hormonal therapy and 52% had ongoing, second-generation antihormonal therapies, such as abiraterone or enzalutamide. Ninety-eight of the men (72%) had undergone prior cytotoxic chemotherapy, and 34 of them (35%) had also undergone second-line chemotherapy.

In total, we administered 487 cycles of PSMA-RLT. The median amount of activity per cycle was 6.2 GBq (range of 3.2-7.5 GBq), and the cumulative amount of activity was 21 GBq in a median of three cycles per patient (range 1-11 cycles). Twelve patients received only one cycle of PSMA-RLT, 11 of whom due to rapid disease progression. The other patients showed a very good response after only one cycle (a 70% PSA decrease); thus, we decided to stop treatment and to follow-up with him instead.

The interval between the cycles was in median 7.5 weeks and varied between 5.2 and 67.3 weeks. The longer periods between the cycles were because of rechallenge therapy in patients with PD who previously benefited from the initial treatment with PSMA-RLT. Most of the patients who received rechallenge cycles PSMA-RLT continued the initial antihormonal treatment in the intervals between the treatments as maintenance therapy. There were only two patients who changed the antihormonal treatment during the rechallenge therapy: the first one received previously abiraterone and then switched to enzalutamide and the second one discontinued enzalutamide.

Final survival analysis was conducted when all of the patients had died, which occurred after a median follow-up of 50 weeks (range 2-177 weeks). The OS correlated positively with the number of administered cycles of PSMA-RLT. Patients who received one to two cycles survived only 19 weeks, which were much shorter than the OS of patients who received three to four cycles (57 weeks OS) or five and more cycles (111 weeks OS). The difference in the univariate and

the multivariate analyses between these categories was significant with $P < .001$. Similarly, also the higher cumulative activity correlated with better OS; $P < .001$ in the univariate and multivariate analyses.

Fourteen of the patients received another treatment after the PSMA-RLT. The consecutive treatment was in most of the cases chemotherapy: docetaxel ($N = 4$) and second-line chemotherapy ($N = 6$). However, the median OS of these patients did not significantly differ from patients who did not receive consecutive treatment: 49 vs 57 months, respectively.

3.2 | Predictive biochemical markers at the baseline

Of the tested biochemical tumor markers, the baseline ALP (with a cut-off of 120 U/L), LDH (with a cut-off of 248 U/L), and PSA (with a cut-off of 47 ng/mL which was first quartile) correlated significantly with survival following PSMA-RLT (Figure 1). Patients with normal ALP (≤ 120 U/L) survived 92 weeks (95% CI, 69.7-114.3), and patients with elevated ALP survived only 37 weeks (95% CI, 32.0-42.0; $P < .001$), with a hazard ratio (HR) of 1.90 (95% CI, 1.2-3.0). Similar results were seen in patients with normal (≤ 248 U/L) and elevated (> 248 U/L) LDH levels: 93 (95% CI, 69.7-114.3) and 37 weeks (95% CI, 32.4-41.6; $P < .001$), with an HR of 2.25 (95% CI, 1.41-3.58). Also, patients with PSA values lower than 47 ng/mL at baseline achieved a longer OS than patients with higher PSA levels: 83 (95% CI, 43.4-122.6) vs 47 weeks (95% CI, 35.8-58.2) with $P = .007$ and HR of 1.97 (95% CI, 1.19-3.27). Nonpredictive baseline tumor markers were CgA (100 ng/mL cut-off) and pro-GRP (63 pg/mL cut-off) (Figure 2).

3.3 | Changes in the tumor markers after the first PSMA-RLT

Univariate analysis (logrank test) and multivariate analysis (Cox proportional-hazards regression model) were conducted to compare favorable responses (tumor marker decrease and stable values) with unfavorable responses (tumor marker increase). To perform the statistics and for more clarity, we have summarized the favorable

responses into one group. More detailed survival data, including parameters regarding values of specific subgroups of patients, are listed in Table 2 and shown in Figure 3. The responses after the first PSMA-RLT of most of the evaluated parameters correlated with the OS. In patients with elevated ALP at the baseline, the OS was 53 weeks (95% CI, 43.2-62.8) in cases with stable or decreased ALP and 28 weeks (95% CI, 21.0-35.0) in cases with increased ALP ($P = .009$; HR, 2.14; 95% CI, 1.18-3.86). A significant difference was also seen between patients with baseline LDH greater than 248 U/L who exhibited stable/decreased LDH (45 weeks, 95% CI, 32.9-57.1) and patients with increased LDH (26 weeks, 95% CI, 5.8-46.2), $P = .005$, and HR of 2.37 (95% CI, 1.26-4.47). Survival in cases of PSA decrease following treatment was 83 weeks (67.5-98.5), and survival in cases of PSA increase was only 26 weeks (95% CI, 18.9-33.1) ($P < .001$; HR, 4.07; 95% CI, 2.68-6.18). The significance of any measured PSA changes (whether decreased or not decreased) was higher than the significance classified according to PCWG3: $P < .001$ vs $P < .003$ and HR, 4.07 vs 1.94, respectively. In the multivariate analysis, PSA nadir (median, 25.5 ng/mL) in patients who showed any PSA decrease and time to PSA nadir (median, 21 weeks) correlated with the OS: $P = .24$ and $P = .003$, respectively. The duration of PSA nadir (maximum elevation, $< 25\%$) was in median 8 weeks and did not correlate with longer OS. A pro-GRP decrease in patients with elevated baseline values resulted in an 85-week OS (95% CI, 57.8-112.2), which was significantly longer than the 39-week OS of the patients who did not experience any pro-GRP decrease (95% CI, 30.0-48.0) ($P = .013$; HR, 2.0; 95% CI, 1.14-3.67).

3.4 | Tumor markers in patients with bone metastases

We tested the value of ALP and BAP in 133 patients with bone metastases treated with PSMA-RLT. Men with stable or decreased ALP following treatment reached an OS of 70 weeks (95% CI, 54.3-85.7). In cases of increased ALP, OS was only 32 weeks ($P < .001$; HR, 2.36; 95% CI, 1.46-3.80). Patients who reached at least stable BAP levels also had significant longer OS than those with increased BAP:

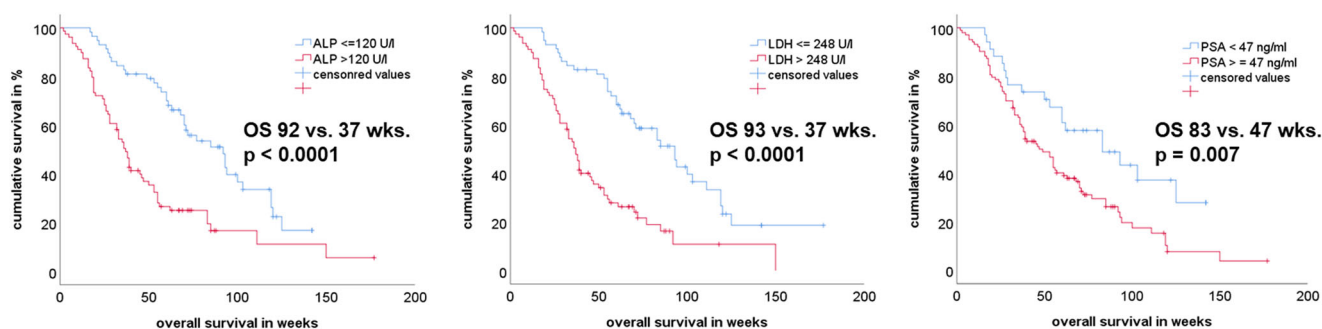


FIGURE 1 Survival analyses (Kaplan-Meier curves) by comparing baseline alkaline phosphatase (ALP) with cut-off 120 U/L, lactate dehydrogenase (LDH) with cut-off 248 U/L, and prostate-specific antigen (PSA) with cut-off 47 ng/mL (first quartile). ALP ≤ 120 vs > 120 U/L: 92 vs 37 weeks, $P < .001$, and hazard ratio, 1.90 (95% CI, 1.2-3.0). LDH ≤ 248 vs > 248 U/L: 93 vs 37 weeks, $P < .001$, and hazard ratio, 2.25 (95% CI, 1.41-3.58). PSA < 47 vs ≥ 47 ng/mL: 83 vs 47 weeks, $P = .007$, and hazard ratio, 1.97 (95% CI, 1.19-3.27). OS, overall survival [Color figure can be viewed at wileyonlinelibrary.com]

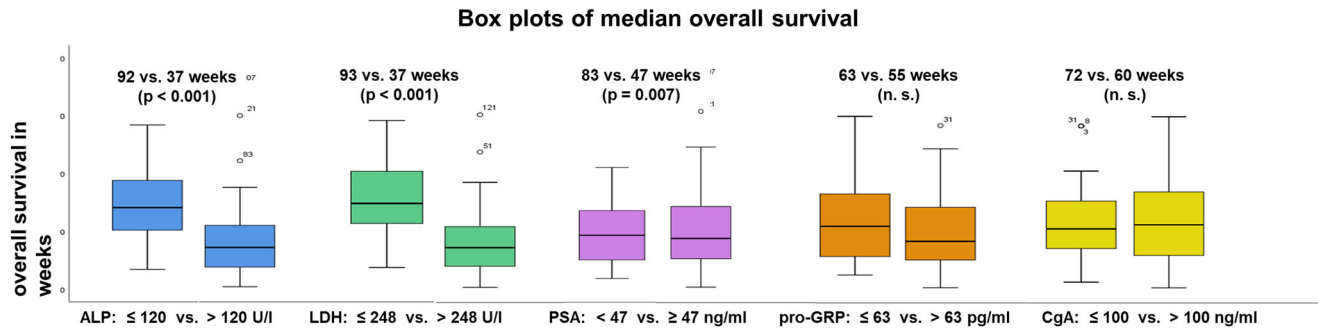


FIGURE 2 Box plots comparing predictive and nonpredictive basal biochemical markers for the overall survival index. ALP, alkaline phosphatase; CgA, chromogranin A; LDH, lactate dehydrogenase; n.s., not significant; pro-GRP, pro-gastrin-releasing peptide; PSA, prostate-specific antigen [Color figure can be viewed at wileyonlinelibrary.com]

72 (95% CI, 48.7-95.3) vs 36 weeks (95% CI, 27.5-44.5) ($P = .002$; HR, 2.06; 95% CI, 1.29-3.28). Figure 5 illustrates the OS of patients with bone metastases according to the changes in their ALP and BAP levels. To compare the value of ALP with BAP, we divided the patients depending on the number of bone metastases into four groups: patients with less than 6 metastases, 6 to 20 metastases, greater than 20 metastases, and superscan. BAP showed in none of the subgroups more superior correlation with OS as compared to ALP.

3.5 | Neuroendocrine tumor markers

Patients with normal CgA levels at the baseline survived 12 weeks longer than those with elevated values (100 ng/mL cut-off): 72 (95%

CI, 45.9-98.1) vs 60 weeks (95% CI, 39.1-80.9). However, this difference was insignificant in our cohort of patients. We also tested the changes in CgA following the treatment of patients with elevated baseline values ($N = 69$). Patients with stable tumor marker levels exhibited the longest survival at 93 weeks (95% CI, 41.2-144.8), followed by patients with decreased CgA at 61 weeks (95% CI, 44.5-77.5). The shortest survival, 30.3 weeks, was seen in patients with increased CgA (95% CI, 0.0-104.4). Again, this difference was not statistically significant.

Neuroendocrine tumor marker pro-GRP (63 pg/mL cut-off) was also not predictive at the baseline; OS was 63 weeks (95% CI, 44.4-81.6) for pro-GRP ≤63 pg/mL and 55 weeks (95% CI, 37.8-72.2) in cases of elevated pro-GRP. However, in patients with elevated baseline pro-GRP ($N = 85$), a significantly longer OS was observed in cases of decreased

TABLE 2 Overall survival rates dependent on tumor marker changes after treatment in all patients and selected subgroups of patients

Median OS in weeks						Significance			
						↓/↔ vs ↑		Any ↓ vs No ↓	
	↓	↔	↑	Any ↓	No ↓	P^1	P^2	P^1	P^2
PSA	77	68	25	83	26	<.001	<.001	<.001	<.001
ALP	150	70	32	77	49	<.001	<.001	.003	.004
ALP ^{sub*}	72	70	32	72	49	<.001	<.001	.011	.013
ALP ^{sub**}	55	46	28	53	33	.009	.012	.008	.011
BAP ^{sub*}	60	77	36	85	50	.002	.002	.004	.004
LDH	56	71	26	56	60	.002	.003	NS	-
LDH ^{sub*}	56	38	26	45	35	.005	.008	.023	.027
CgA	61	93	30	60	83	NS	-	NS	-
CgA ^{sub*}	61	93	45	60	92	NS	-	NS	-
pro-GRP	55	55	39	71	50	NS	-	NS	-
pro-GRP ^{sub*}	55	55	39	85	39	NS	-	.013	.016

Abbreviations: ALP, alkaline phosphatase; ALP^{sub*}, alkaline phosphatase in patients with bone metastases; ALP^{sub**}, alkaline phosphatase in patients with baseline values greater than 120 U/L; Any ↓, any tumor marker decrease; BAP^{sub*}, bone-specific alkaline phosphatase in patients with bone metastases; CgA, chromogranin A; CgA^{sub*}, chromogranin A in patients with baseline values greater than 100 U/L; LDH, lactate dehydrogenase; LDH^{sub*}, lactate dehydrogenase in patients with baseline values greater than 248 U/L; No ↓, no tumor marker decrease; NS, not significant; OS, overall survival; P^1 , P value from the logrank test; P^2 , P value from the Cox proportional-hazards regression analysis; pro-GRP, pro-gastrin-releasing peptide; pro-GRP^{sub*}, pro-gastrin-releasing peptide in patients with baseline values greater than 63 pg/mL; PSA, prostate-specific antigen; ↓, decrease: if ≥50% decrease for PSA and if ≥25% decrease for ALP, BAP, LDH, CgA, and pro-GRP; ↔, stable: if less than 50% decrease or less than 25% increase for PSA and if less than 25% increase or decrease for ALP, BAP, LDH, CgA, and pro-GRP; ↑, increase: if ≥25% increase for PSA, ALP, BAP, LDH, CgA, and pro-GRP.

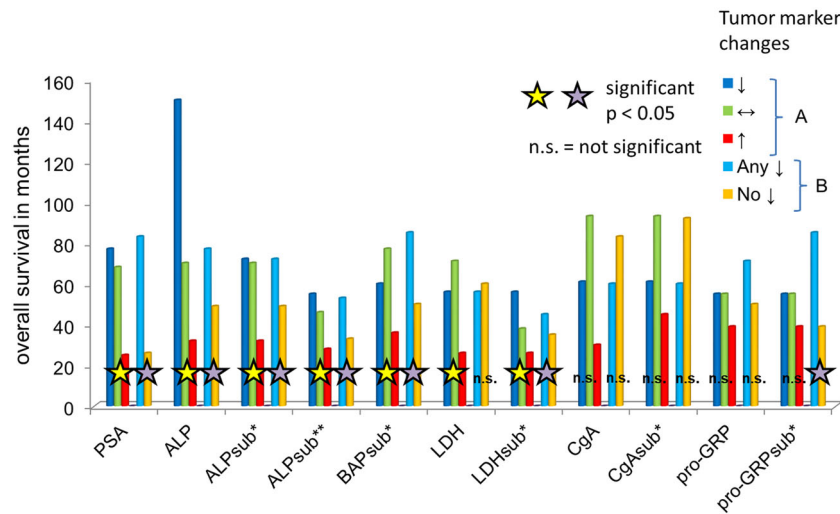


FIGURE 3 The difference in the overall survival dependent on tumor marker changes after treatment in all patients and selected subgroups of patients. The significance of the difference was tested between patients: A, with tumor marker decrease and stable values ($\downarrow/\leftrightarrow$) vs those with tumor marker increase (\uparrow), marked with yellow stars in case of $P < .05$. B, With any tumor marker decrease (Any \downarrow) vs those with no tumor marker decrease (No \downarrow), marked with purple stars in case of $P < 0.05$. ALP, alkaline phosphatase; ALP^{sub+} alkaline phosphatase in patients with bone metastases; ALP^{sub**} alkaline phosphatase in patients with baseline values greater than 120 U/L; Any \downarrow , any tumor marker decrease; BAP^{sub+}, bone-specific alkaline phosphatase in patients with bone metastases. CgA, chromogranin A; CgA^{sub+}, chromogranin A in patients with baseline values greater than 100 U/L; LDH lactate dehydrogenase; LDH^{sub+}, lactate dehydrogenase in patients with baseline values greater than 248 U/L; No \downarrow , no tumor marker decrease; \leftrightarrow , stable: if less than 50% decrease or less than 25% increase for PSA and if less than 25% increase or decrease for ALP, BAP, LDH, CgA, and pro-GRP; \uparrow , increase: if $\geq 25\%$ increase for PSA, ALP, BAP, LDH, CgA, and pro-GRP [Color figure can be viewed at wileyonlinelibrary.com]

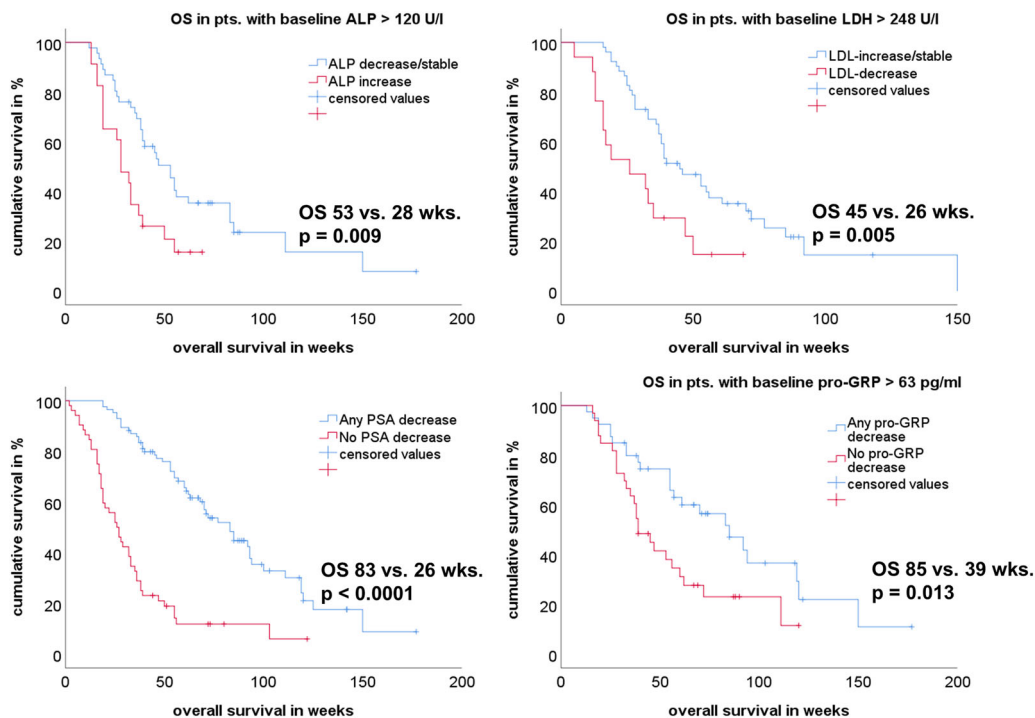


FIGURE 4 Survival analyses (Kaplan-Meier curves) of selected groups of patients with prostate cancer patients who underwent PSMA-RLT. Top-right graph: correlation between the overall survival (OS) and changes in the alkaline phosphatase (ALP) levels of patients with elevated baseline ALP (>120 U/L). Top-left graph: correlation between the OS and changes in the lactate dehydrogenase (LDH) levels of patients with elevated baseline LDH (>248 U/L). Bottom-right graph: correlation between the OS and changes in prostate-specific antigen (PSA). Bottom-left graph: correlation between the OS and changes in the pro-gastrin-releasing peptide (pro-GRP) of patients with elevated baseline pro-GRP (>63 pg/mL). PSMA-RLT, prostate-specific membrane antigen-radioligand therapy [Color figure can be viewed at wileyonlinelibrary.com]

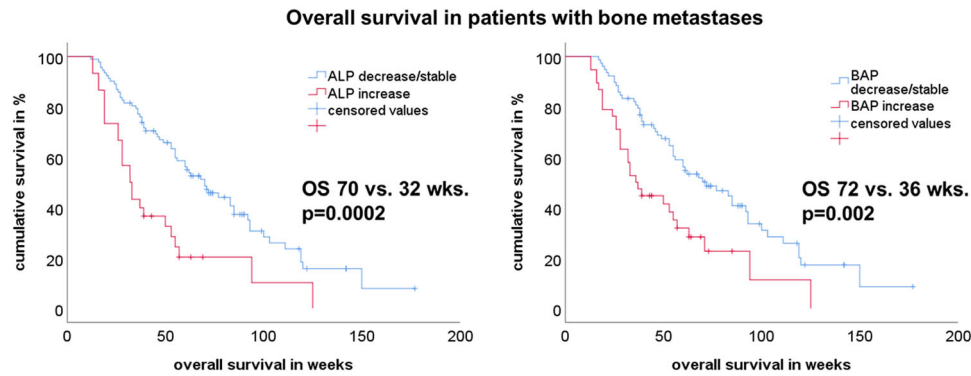


FIGURE 5 Survival analyses (Kaplan-Meier curves) after PSMA-RLT in patients with bone metastases from prostate cancer. Right graph: correlation between overall survival (OS) and changes of alkaline phosphatase (ALP). Left graph: correlation between OS and changes of bone-specific alkaline phosphatase (BAP). PSMA-RLT, prostate-specific membrane antigen-radioligand therapy [Color figure can be viewed at wileyonlinelibrary.com]

pro-GRP following PSMA-RLT: 85 (95% CI, 57.8-112.2) vs 39 weeks in cases with no pro-GRP decrease (95% CI, 30.0-48.0) ($P = .013$; HR, 2.05; 95% CI, 1.14-3.67). This is visualized in Figure 4 (bottom right).

3.6 | Kinetic patterns of different tumor markers

Similar to the responses after the first-cycle PSMA-RLT, the kinetic patterns of the tumor markers ALP, BAP, LDH, and PSA also showed significant correlation with the OS. In summary, best survival achieved the patients with steadily declining, stable, or mixed responses (first decreasing/stable, then increasing or first increasing, then decreasing/stable) of the mentioned tumor markers and the worse survival patients with steadily increasing values. However, it is to mention that the cohorts with mixed kinetics were rather small and heterogeneous. Included in these groups were patients who received a continuous PSMA-RLT as well as patients with intermittent therapy with long treatment pauses. Table 3 gives an overview of the correlation between the OS and the kinetic patterns of the different tumor markers.

4 | DISCUSSION

Currently, PSMA-RLT is considered a last-line treatment option in advanced PC, which means that patients who start the treatment already have metastatic CRPC and, in most cases, are symptomatic.^{16,17} Thus, accurate estimation of OS is essential to the management of possible treatment options. Patients with CRPC are not a homogeneous group, as their survival can range from several weeks to several years. Therefore, precise tests that can distinguish whether treatment would be beneficial are needed to avoid the loss of valuable time and unnecessary side effects.³⁴ According to some authors, PSA is not a reliable marker for monitoring CRPC.^{35,36} Furthermore, Scher et al³⁷ postulated that PSA changes 4, 8, and 12 weeks following chemotherapy are not predictive for survival following first-line chemotherapy. Nevertheless, due to the lack of precise alternative tests, PSA changes

are the basis for important therapeutic decisions, such as whether to start secondary or tertiary hormonal interventions.³⁴

In our study, PSA changes correlated significantly with OS following PSMA-RLT. Interestingly, in our patient cohort, all PSA decreases following treatment showed a stronger correlation with the OS than PSA changes of at least 50%. One explanation for this is that the correlation between PSA and survival might have been biased, as the decisions of whether to continue treatment were based on PSA changes. Thus, in accordance with Rahbar et al²⁴ and results of the current study, the cumulative administered activity of PSMA-RLT also seemed to correlate positively with survival. Similarly, other reports have suggested that all PSA decreases are reliable markers for predicting response and survival following PSMA-RLT.^{20,24,31}

The results of a large meta-analysis showed that elevated ALP at the baseline is associated with short progression-free survival and poor OS in men with PC. Thus, ALP is a reliable and, due to its cost effectiveness, convenient prognostic biomarker.³⁸ Furthermore, ALP is associated with PSA responses in patients following chemotherapy.³⁹⁻⁴² ALP was also tested as an independent predictive marker for the survival of patients who undergo PSMA-RLT.²²⁻²⁵ In the current study, normal ALP values (≤ 120 U/L) exhibited better OS than abnormal ALP values ($P < .001$; HR, 1.90; 95% CI, 1.2-3.0). Patients with elevated ALP at the baseline could have longer OS in the case of a decrease or stabilization of the marker ($P = .009$; HR, 2.14; 95% CI, 1.18-3.86).

The metabolism of cancer cells shifts to aerobic glycolysis with the production of lactate, which is formed from the reduction of pyruvate using LDH. Therefore, the serum level of LDH is often higher in patients with malignant tumors. High-serum LDH is associated with poor survival in patients with solid tumors and particular melanomas, prostate, and renal cell carcinomas, and it can be a useful and inexpensive prognostic biomarker for metastatic carcinomas.⁴³ Several studies have confirmed the predictive value of LDH in patients with CRPC who undergo chemotherapy.^{37,39,41,43,44} However, in previous reports made by our study group, LDH seemed not to correlate with the PSA responses and survival of patients treated with PSMA-RLT.^{31,32} In this study, we evaluated and followed up with 137 patients from their PSMA-RLT until their deaths, and we

TABLE 3 Overall survival rates dependent on kinetic patterns of tumor markers in all patients and selected subgroups of patients

Marker	Median OS in weeks					Significance <i>p</i> (χ^2)
	steadily↓	↔	steadily↑	first ↓/↔ then ↑	first ↑ then ↓/↔	
PSA	83	37	32	71	22	<.001
ALP	nr	83	32	63	94	<.001
ALP ^{sub*}	83	77	32	63	94	<.001
ALP ^{sub**}	55	40	32	45	nr	.041
BAP ^{sub*}	60	70	33	93	37	.005
LDH	70	93	19	39	33	<.001
LDH ^{sub*}	70	39	19	38	32	.015
CgA	68	83	83	57	103	NS
CgA ^{sub*}	85	60	40	61	111	NS
pro-GRP	61	70	39	70	83	NS
pro-GRP ^{sub*}	83	55	39	62	39	NS

Abbreviations: ALP, alkaline phosphatase; ALP^{sub*}, alkaline phosphatase in patients with bone metastases; ALP^{sub**}, alkaline phosphatase in patients with baseline values greater than 120 U/L; BAP^{sub*}, bone-specific alkaline phosphatase in patients with bone metastases; CgA, chromogranin A; CgA^{sub*}, chromogranin A in patients with baseline values greater than 100 U/L; LDH, lactate dehydrogenase; LDH^{sub*}, lactate dehydrogenase in patients with baseline values greater than 248 U/L; nr, not reached; NS, not significant; OS, overall survival; pro-GRP, pro-gastrin-releasing peptide; pro-GRP^{sub*}, pro-gastrin-releasing peptide in patients with baseline values greater than 63 pg/mL; PSA, prostate-specific antigen; steadily ↓, steadily decreasing tumor marker values; ↔, stable tumor marker values; steadily ↑, steadily increasing tumor marker values; first ↓/↔ then ↑, first decreasing/stable then increasing tumor marker values; first ↑ then ↓/↔, first increasing then decreasing/stable tumor marker values.

observed a significant correlation between elevated baseline LDH (>248 U/L) and shorter OS ($P < .001$; HR, 2.25; 95% CI, 1.41-3.58). A significant difference in OS was also seen between patients with initially elevated LDH who reached stable or decreased amounts and patients with further increases in LDH following treatment (45 vs 26 weeks, respectively) ($P = .005$; HR, 2.37; 95% CI, 1.26-4.47).

Bones remain one of the main sites in which advanced PC spreads, as more than two-thirds of men with PC develop bone metastasis.⁴⁵ Bone metastases weaken the structure of the skeleton, which can result in pain and disabling skeletal-related events, such as pathologic fractures or spinal cord compression. Other common effects include ineffective hematopoiesis and hypercalcemia.^{46,47} Skeletal-related events are associated with a decreased quality of life and short OS.^{48,49} Elevated serum BAP in patients with CRPC with bone metastases is associated with a higher risk of adverse skeletal events occurring.⁵⁰ However, BAP does not seem to be prognostic of the survival of patients treated with chemotherapy.⁵¹ The relevance of BAP changes in patients, who underwent PSMA-RLT, has not been reported in previous studies. In our study, patients who reached at least stable BAP values following PSMA-RLT had significantly longer OS than those with increased BAP: 72 vs 36 weeks ($P = .002$; HR, 2.06; 95% CI, 1.29-3.28). Similarly, men with bone metastases and stable or decreased ALP following treatment achieved significantly longer OS ($P < .001$; HR, 2.36; 95% CI, 1.46-3.80). In our cohort of patients, BAP could not show more superior correlation with the OS compared to ALP.

Initially, most PCs are androgen-sensitive; however, after each relapse, tumors become more undifferentiated and become hormone-resistant. At this stage of the disease, the appearance of neuroendocrine cells is not uncommon. The prevalence of neuroendocrine cells in patients with PC is about 10%; however, in advanced

cancer, it is 31%. Peptides and growth factors from neuroendocrine-differentiated prostate cells promote tumor growth and apoptosis inhibition.^{52,53} Furthermore, several studies have concluded that neuroendocrine differentiation is associated with hormone resistance due to its lack of androgen receptors and correlates significantly with poor survival; however, these data are controversial.⁵⁴⁻⁵⁹ A patient's last biopsy occurs at the time of diagnosis before they have even started their hormonal therapy; thus, the biopsy is not necessarily representative of the castration-resistant stage of the disease. In patients with metastatic cancer that includes bone metastases or pelvic lymph node metastases, it is not easy to biopsy to obtain a sample.³⁴ In such cases, PSA might not be a reliable tumor marker.⁶⁰ Therefore, neuroendocrine tumor markers measured in serum such as CgA and pro-GRP may be useful for supplying more information about both the neuroendocrine differentiation and the prognosis. The clinical value of CgA and pro-GRP has been evaluated in several studies, which demonstrated that prognostic significance is an independent marker in PC.⁶⁰⁻⁶² Furthermore, neuroendocrine markers can guide treatment sequence selections, for example, in the case of abiraterone or docetaxel.⁶³ Still, inconsistent data exist as to whether neuroendocrine markers in patients with CRPC correlate with PSA response.^{59,64} The relevance of neuroendocrine tumor markers in patients who undergo PSMA-RLT has not been evaluated in previous reports. The current study showed that men with abnormally high CgA but stable or decreased CgA levels following treatment survived longer than those with increased CgA. However, this difference was not statistically significant. In contrast, patients with elevated baseline pro-GRP exhibited significantly higher OS when their pro-GRP levels declined following PSMA-RLT than in the cases in which they did not: 85 vs 39 weeks ($P = .013$; HR, 2.05; 95%

CI, 1.14-3.67). The reason why pro-GRP correlated more with OS than CgA may have been the highly aggressive nature of tumors at this stage of PC. Elevated pro-GRP has been observed particularly in patients with distant metastases and hormone-resistant PC or short response durations following antihormonal therapy.^{52,65-67}

The prognostic values of the PSA kinetics after different treatments have been studied in various studies.^{68,69} However, to our knowledge there is no published data about the correlation between OS and changes of the ALP, BAP, LDH, and neuroendocrine tumor markers after PSMA-RLT over time. Our results show that the kinetic patterns of tumor marker are also important factors to predict the OS of treated patients. Especially, the kinetics of the markers PSA, ALP, BAP, and LDH showed significant correlation with the survival. Patients with steadily decreasing or stable values achieved a significantly longer OS than patients with steadily increasing markers. Some patients showed mixed kinetics of the tumor markers: first increasing/stable, then increasing or first increasing, then decreasing/stable. These patients also survived longer than patients with steadily increasing tumor marker. However, these cohorts were small and very heterogeneous, because some of the patients were under continuous treatment with PSMA-RLT and other patients had long treatment pauses after a good response.

This single-center study in Germany included 137 patients who received a total of 487 cycles of PSMA-RLT between January 2015 and November 2017. A possible limitation of this study is that since all the data were collected retrospectively, no defined criteria were used for the patient selection, and the lack of standardization may have induced bias. Furthermore, the correlation between PSA and survival in our study may be biased because the decisions regarding whether to continue the patients' treatment were based on the PSA changes. Several strengths of the study, however, include a large number of patients and the long follow-up duration since all patients were followed up until death. This data can be used to plan future prospective studies that evaluate OS following PSMA-RLT.

5 | CONCLUSION

In addition to the established tumor marker PSA, a steady state of and/or decrease in most of the evaluated parameters in the selected patients who underwent PSMA-RLT—ALP, LDH, PSA, pro-GRP, and BAP—were associated with significantly longer OS in the univariate and multivariate analyses.

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CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

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