Aus dem Institut für Gewebediagnostik Berlin am MVZ HELIOS Klinikum Emil von Behring

DISSERTATION

HISTOPATHOLOGISCHE ASPEKTE DER KRYOBIOSIE DER LUNGE

(Einfluss der Art der Materialgewinnung auf die Diagnosefindung in der Lungenpathologie)

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Inhalt

ABSTRAKT

Das Ziel der Arbeit ist die Darstellung der Möglichkeiten der Kryobiopsien für die Diagnostik von Lungenerkrankungen und Pleuraerkrankungen. Es wurde zunächst gezeigt, dass die transbronchialen Kryobiopsien einen deutlichen morphologischen Unterschied gegenüber den konventionellen transbronchialen Biopsien zeigen, indem sie größer sind und häufiger alveoläres Gewebe enthalten. Entnahmebedingte Artefakte des alveolären Gewebes zeigten sich nicht. Untersucht wurden Patientenkollektive mit Krebserkrankungen sowie mit interstitiellen Lungenerkrankungen. In beiden Gruppen zeigt sich eine deutliche Steigerung der diagnostischen Ausbeute. Bei den pleuralen Läsionen konnte mindestens eine Gleichwertigkeit der Methode der Kryobiopsie der Pleura mit einer flexiblen Zangenbiopsie gezeigt werden. Die letzte vorgelegte Arbeit zeigt die erste Beschreibung einer durch Kryobiopsie gesicherten diffusen idiopathischen Hyperplasie der neuroendokrinen Zellen (DIPNECH). Insgesamt zeigt sich eine gute Einsetzbarkeit der Methode bei allen Lungen- und Pleuraerkrankungen.

ABSTRACT

The aim of this work is to present a variety of cryobiopsies for the diagnosis of pulmonary and pleural diseases. The first paper describes a distinct morphologic difference between cryobiopsies and traditional transbronchial biopsies. Cryobiopsies reveal to be larger, contain more common alveolar tissue, which does not show any artefacts. The following analyses included patient groups with cancer and interstitial lung diseases. Both groups show a higher diagnostic yield concerning the specific disease. For the pleural lesions it could be demonstrated that there is at least a diagnostic equivalence between cryobiopsy and flexible thoracosopy. Finally, the last paper is a first case report of a cryobiopsy-diagnosed diffuse idiopathic neuroendocrine cell hyperplasia (DIPNECH). Altogether, the method presents itself suitable for application in all groups of lung and pleural diseases.

EINFÜHRUNG

Die Einführung der flexiblen Zangenbiopsie in der bioptischen Diagnostik der Lungenerkrankungen bedeutete einen enormen Fortschritt im medizinischen Alltag. Bisher unerreichbare Gebiete der Lunge wurden plötzlich endoskopisch einsehbar und damit unter anderem für eine histopathologische Diagnostik zugänglich (1,2). Schnell hat sich jedoch

herausgestellt, dass auch diese grundsätzlich an sich sichere und gut zu etablierende Methode ihre Grenzen hat. Diese liegen in erster Linie in der Größe der entnommenen Gewebeproben. In den letzten Jahren hat sich die Größe der eingesetzten Instrumente kaum verändert. Ähnlich blieb daher auch die diagnostische Ausbeute dieser Methode: große Fallstudien berichten über diagnostische Ausbeute von 50% bis 70% in Abhängigkeit von der Indikation, Größe und Lokalisation der Läsionen (2,3,4,5,6,7,8). In diffusen, interstitiellen Lungenerkrankungen (ILE) sind die Ergebnisse unselektiert (für die gesamte Gruppe) wahrscheinlich schlechter (8–12). Andererseits, bei ILE mit Herdbefund oder Herdbefunden wie z.B. Sarkoidose oder organisierender Pneumonie (OP) sehr gut (13). Die Resultate bei gewöhnlicher interstitieller Pneumonie (usually intertitial pneumonia/UIP) oder pulmonaler Histiozytose sind hingegen deutlich ernüchternder (14,15,16). Die Daten zur bioptischen Diagnostik der Pneumokoniose sind sehr rar, lassen jedoch hypothetisch schlechte Ergebnisse vermuten. Der Hauptgrund dieser diagnostischen Insuffizienz scheint der Mangel an alveolarem Gewebe zu sein. Daraufhin wurde von Fraire et al. eine diagnostische Mindestanzahl von 20 Alveolen pro Biopsie vorgeschlagen (17). Trotzdem bleibt die offene Lungenbiopsie, verglichen mit traditionellen bronchoskopischen Methoden, die Methode der Wahl in der histologischen Diagnostik der ILE (9,18,19,20).

Bei Lungentumoren sind die Ergebnisse viel besser und zeigen in der gesamten Gruppe ein relativ gutes Ergebnis von über 70 % im einsehbaren Bereich (4,5,6,7). Die Gewebsstücke sind in der Regel sehr klein (1–2 mm). Entnahmeartefakte können unter Umständen eine exakte histopathologische Diagnose erschweren. Bei dieser geringen Materialmenge ist es nicht ausgeschlossen, dass das Tumorgewebe am Ende für eine molekularpathologische Diagnostik nicht ausreicht. Dieser Aspekt gewinnt, insbesondere in palliativen Fällen, immer mehr an Bedeutung. Daher wird die Zangenbiopsie sehr oft mit anderen Methoden (z.B. Bürstenzytologie, Nadelpunktion sowie Bronchiallavage) kombiniert, diese werden jedoch häufig in der Form von Ausstrichen geliefert und sind daher für eine Immunohistochemie oder Immunozytochemie nur eingeschränkt verwendbar. Es bleibt jedoch eine nicht kleine Gruppe von Patienten übrig, bei denen die konventionelle Zangenbiopsie zu frustranen Ergebnissen führt, zudem sind diese Lokalisationen für eine transthorakale Stanzbiopsie häufig ungeeignet. Diese Patienten müssen dann in der Regel einer chirurgischen, eventuell einer intraoperativen Schnellschnittdiagnostik zugeführt werden.

WISSENSSTAND UND LITERATURDATEN ZUM ZEITPUNKT DES ARBEITSBEGINNS

Die Technik der heutigen Kryobiopsie geht mit ihren Anfängen in die 60-er Jahre zurück (21). Das allgemeine Prinzip der Probeentnahmen basiert auf Gewebsadhäsion an der Sondenspitze bei tiefer Kühlung derselben. Die Kühlung wird durch den so genannten Joule-Thomson-Effekt, meistens durch CO^2-Gabe, erreicht. Auf Grund der fehlenden flexiblen Technik der Bronchoskopie wurde die Methode initial nur für die palliative Behandlung stenosierender Tumore der zentralen Anteile des Bronchialsystems eingesetzt. Nach der Etablierung der flexiblen Endoskopietechnik wurde das Verfahren ab Ende der 1990er Jahre zunächst wieder für die Rekanalisation verwendet (22–24), ab ca. 2000 wurden die ersten Versuche unternommen, diese Methode auch zu diagnostischen Zwecken zu nutzen. Diese Arbeiten bezogen sich sowohl auf Lungentumoren, als auch auf interstitielle Lungenerkrankungen. So konnten Babiak et al. bei einer Gruppe aus 41 Patienten eine deutliche Steigerung der diagnostischen Ausbeute bei diffusen Lungenerkrankungen zeigen (25). Auch bei Lungentumoren konnte ein deutlicher diagnostischer Zugewinn gezeigt werden (26,27). Dabei zeigte sich keine Steigerung der relevanten untersuchungsbedingten Komplikationen (24,25,26,28).

Auch im Bereich der intrathokakalen Intervention wurden bereits Versuche mit der Kryosonde unternommen (29). In dieser Arbeit von Rozman et al. wurde die Methode als sicher und routinetauglich beschrieben, allerdings wurde das Verfahren bisher nicht mit den anderen Methoden (starre und flexible Thorakoskopie) verglichen.

DIE LISTE DER VORGELEGTEN ARBEITEN

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2. Grüning W, Ammenwerth W, Wurps H, Kollmeier J, Blum T, Schönfeld N, Griff S, Bauer TT.: Diagnostischer Wert und Sicherheit der Bronchoskopischer Kryotechnik im Routineeinsatz bei Verdacht auf Lungenkarzinom. Pneumologie. 2013 Dec; 67(12):676-82. doi: 10.1055/s-0033-1344853.

3. Griff S, Schönfeld N, Ammenwerth W, Blum TG, Grah C, Bauer TT, Grüning W, Mairinger T, Wurps H.: Diagnostic yield of transbronchial cryobiopsiy in non-neoplastic lung disease: a retrospective case series. BMC Pulm Med. 2014 Nov 3;14:171. doi: 10.1186/1471-2466-14-171.
4. Wurps H, Schönfeld N, Bauer TT, Bock M, Duve C, Sauer R, Mairinger T, Griff S.: intra-patient comparison of parietal pleural biopsies by rigid forceps, flexible forceps and cryoprobe obtained during medical thoracoscopy: a prospective series of 80 cases with pleural effusion. BMC Pulm Med. 2016 Jul 7; 16(1):98. doi: 10.1186/s12890-016-0258-5.
5. Sauer R, Griff S, Blau A, Franke A, Mairinger T, Grah C.: Diffuse idiopathic pulmonary neuroendocrine cell hyperplasia diagnosed by transbronchial lung cryobiopsy: a case report. J Med Case Rep. 2017 Apr 7; 11(1):95. doi: 10.1186/s13256-017-1254-y.

Das Ziel der vorgelegten kumulativen Arbeit war, den diagnostischen Wert der Kryobiopsie bei verschiedenen Erkrankungen und Fragestellungen der Pneumologie, insbesondere im Vergleich zu den traditionellen Methoden (in 1. Linie zur konventionellen Zangenbiopsie), festzustellen. Wenn man die 5 vorgelegten und bereits publizierten Studien als Teile einer Arbeit betrachtet, werden 2 Forschungsaspekte klar:

1. Morphometrie als solche, um die Mengen des untersuchten Gewebes zu definieren und mit bereits bestehenden endoskopischen Methoden zu vergleichen. Sowohl in der Arbeit 1 als in der Arbeit 4 wird versucht, diese Fragen zu beantworten, wenngleich in der Arbeit 1 nur morphometrische Aspekte beleuchtet werden, während in der Arbeit 4 das eigentliche Ziel eine Verbesserung der diagnostischen Ausbeute war (s.u.). In diesen wurden auch Kriterien festgelegt, die sowohl aus unserer Sicht als auch nach Angaben der Literatur ein Qualitätsmerkmal hinsichtlich der diagnostischen Aussagekraft bei jeweiliger Fragestellung sein sollen. In der Arbeit 2 wurden solche Kriterien nicht herausgearbeitet, da das Wuchsmuster der Tumore sehr unterschiedlich ist und sie, ungeachtet des histologischen Typs, allgemein gesehen, nicht an eine anatomische Struktur gebunden sind, die eine Diagnose erst ermöglicht (wie z.B. alveoläres Gewebe bei ILE oder subpleurales Fett bei Mesotheliom).

2. Diagnostischer Wert der Untersuchungen mit der Kryobiopsie, und zumindest teilweiser Vergleich zu konventionellen Untersuchungsmethoden, sofern dies ethisch vertretbar war. So wurde in der Studie über interstitielle Lungenerkrankungen neben der Kryobiosie keine Zangenbiopsie durchgeführt, da die geringe Ausbeute letzter Methode bei interstitiellen Lungenerkrankungen aus der Literatur bekannt ist. Dieser Forschungsaspekt wird in den Arbeiten 2,3 und 4 deutlich. Die 5. Arbeit als case report ist auch unter dieser Kategorie zu sehen, wenngleich bei einer solchen Art von Publikationen keine generellen Schlussfolgerungen zu ziehen sind.

MATERIAL UND METHODEN

Alle 4 Studien basieren auf routinemäßigen Untersuchungen im Rahmen der täglichen Diagnostik, wobei es sich in der Arbeit 1 und Arbeit 3 um eine retrospektive Analyse und in den beiden anderen Arbeiten um eine jeweils prospektive Analyse handelt. Von jedem eingeschlossenen Patienten lag eine Einverständniserklärung vor. Alle Voraussetzungen für eine invasive Bronchoskopie (EKG, Gerinnnungsdiagnostik, Lungenfunktion, suffiziente Bildgebung, gegebenenfalls fristgerechtes Absetzen von Antikoagulantien) wurden erfüllt. Als klare Ausschlusskriterien dienten eine Thrombozytopenie von weniger als 100000/ml und radiologische Nähe der zu untersuchenden Läsion (sofern es sich um einen Herdbefund handelte) zu großen Blutgefäßen bei Untersuchungen der Lunge. Über die Vereinbarkeit der Untersuchung mit der Komorbidität des jeweiligen Patienten wurde nach Ermessen der behandelnden Pneumologen entschieden. Die Untersuchungsbedingungen waren in allen Fällen leitliniengerecht.

Probenentnahme: Bronchoskopien

Es wurde überwiegend die flexible Bronchoskopie eingesetzt, nur in einem Teil der Fälle in der Arbeit 2 kam ein starres Bronchoskop zum Einsatz. Zu den eingesetzten Instrumenten zählen: Videobronchoskopie (Olympus GmbH, Hamburg, Deutschland) und starre Bronchoskopie (Karl Storz GmbH, Tübingen, Deutschland).

Im Bereich der vergleichenden Morphometrie wurde im Rahmen der konventionellen transbronchialen Biopsie die 1,8 mm-Zange und zum Vergleich des diagnostischen Zugewinns bei malignen Tumoren die Zangen von 2,0 und 2,8 mm eingesetzt (alle Boston Scientific Radial Jaw Natick, MA, USA).

Für die Probeentnahmen wurden unterschiedliche Kryosonden (alle ErbokryoERBE Medizintechnik, Tübingen, Deutschland) verwendet:

Für die zentralen Entnahmen:	780 mm lang, 2,4 mm im Durchmesser
Für die peripheren Entnahmen:	780 mm lang, 1,9 mm im Durchmesser
	900 mm lang, 1,9 mm im Durchmesser

Die Kühlung betrug -77° bis -79° C an der Sondenspitze. Die Entnahme erfolgte durch Platzierung der Sonde an der Läsion oder in Bereich der zu untersuchenden interstitiellen Veränderungen und Zug des Bronchoskops nach einer Frierzeit von 4-5 Sekunden. Nach Abtauen des Gewebes erfolgte die Fixation im 4 % gepufferten Fomalin mit konventioneller Laborbearbeitung und anschließenden Färbungen mittels Hämatoxylin-Eosin (HE), PAS und Elastika-Van-Gieson (EVG) als Standardprogramm im Rahmen der Routinediagnostik. Zur Vermeidung einer inkompletten Erfassung des Gewebes wurden bei allen Proben Stufenschnitte angefertigt. Bei Verdacht auf Malignität bzw. zwecks Tumortypisierung wurden die notwendigen immunohistochemischen Färbungen durchgeführt.

Probenentnahme: Pleurale Biopsien

Mithilfe eines starren Thorakoskops (11 mm, Karl Storz GmbH, Tübingen, Deutschland) wurden Proben durch verschiedene Techniken entnommen:

- Starre Zange (Durchmesser 3 mm, (Karl Storz GmbH, Tübingen, Deutschland).
- Flexible Zange (Durchmesser 2,8 mm, Scientific Radial Jaw Natick, MA, USA)
- Kryosonde 780 mm lang 2,4 mm im Durchmesser, ErbokryoERBE Medizintechnik, Tübingen, Deutschland)

Die Kühlung und die Probenentnahmen sowie Laborbearbeitung wurden analog wie bereits oben beschrieben durchgeführt.

Morphometrische Untersuchungen

Morphometrische Untersuchungen wurden in der Arbeit 1 und 4 durchgeführt. Dafür wurden HE-Schnitte im ZEISS-MIRAX Midi Slide System digitalisiert (MIRAX Viewer Image Software Version 1.12; Zeiss Mikroimaging, Oberkochen, Deutschland und 3D Tech, Budapest, Ungarn). Die entsprechenden Areale (siehe unten) wurden interaktiv manuell markiert und automatisch gemessen. Alle Messungen erfolgten in mm^2.

Gemessen wurden:

In der Arbeit 1: gesamte Fläche des Gewebsstücks sowie alveolarer Anteil der Biopsie. Weiterhin erfolgte die semiquantitative Einschätzung der entnahmebedingten Artefakte.

In der Arbeit 4: gesamte Fläche des Gewebsstücks. Weiterhin erfolgte die Feststellung der sogenannten adäquaten Tiefe und Qualität der jeweiligen Biopsie, wobei als Kriterium das Vorhandensein vom Fettgewebe galt (siehe Diskussion).

In der Arbeit 3 wurden lediglich die linearen Maße der Biopsien aus dem pathologischen Bericht übernommen.

Statistische Analyse

Es wurde mit Statistical Package for Social Sciences, Version 14.0 und 22.0 (SPSS, Chicago, IL, USA) gearbeitet.

ERGEBNISSE

Arbeit 1

Der Durchmesser der Kryoproben war signifikant größer als der der Zangenbiopsien (17,1 vs. 3,8 mm^2).

Der Anteil des miterfassten Alveolargewebes war in der Gruppe der Kryobiopsien größer (73 vs. 56%), wenngleich nicht statistisch signifikant (p=0,290)

Auch der Anteil des Alveolargewebes war, soweit vorhanden, signifikant größer in den Kryobiopsien (11,6 vs.1,9 mm^2).

Auch zeigten Kryoproben keine entnahmebedingten Artefakte des Lungenparenchyms, während in allen Zangenbiopsien diese in unterschiedlichen Schwerestufen zu sehen waren (sofern alveoläres Lungenparenchym miterfasst wurde).

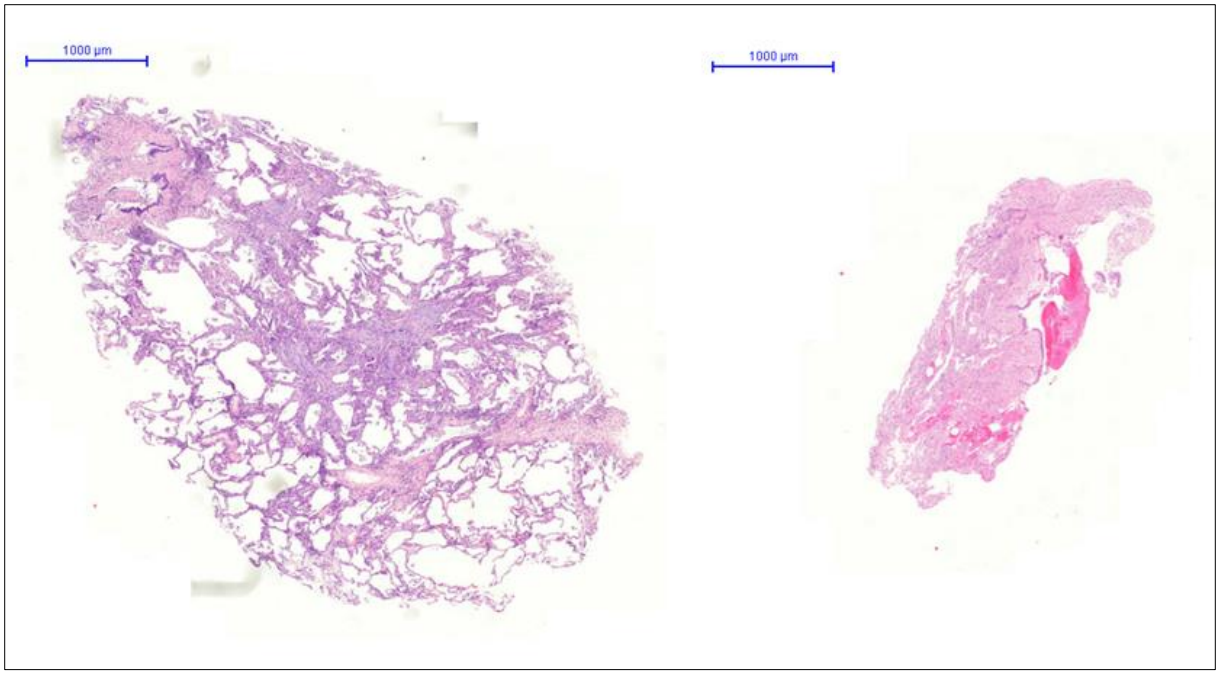

Abb. 1: Morphologischer Vergleich der Kryobiopsie (links) und konventioneller Zangenbiopsie.

Arbeit 2

Bei dem Vergleich des diagnostischen Zugewinns in einer gemischten Gruppe primärer Lungenkarzinome zeigte sich ein statistisch signifikanter diagnostischer Vorteil der Kryobiopsie gegenüber der herkömmlichen Zangenbiopsie bei zentralen Tumoren von 21,5% und bei peripheren Tumoren von 28,5 %. Beim Vergleich der Kombination aller eingesetzten Methoden mit zusätzlicher Kryobiopsie ergibt sich ein diagnostischer Vorteil von 7,4% (p=0,02) bei zentralen und von 19,3 % (p=0,002) bei peripheren Befunden.

Arbeit 3

In der 3. Arbeit wurden die Ergebnisse der diagnostischen Maßnahmen mittels Kryobiopsie bei interstitiellen Lungenerkrankungen ausgewertet und mit Daten der Literatur hinsichtlich der Zangenbiopsie verglichen. Dabei zeigte sich ein deutlicher diagnostischer Zugewinn der hier zu favorisierenden Methode.

Endgültige Diagnose	Anzahl der Fälle	Histologische Sicherung	Literatur-daten
Kryptogen-organisierende Pneumonie (COP)	9	8/9 (89%)	65%
Rheumalunge	2	2/2 (100%)	
Sarkoidose	12	10/12 (83%)	69%
Alveolare Mikrolithiasis	1	1/1 (100%)	
Nicht spezifische interstitielle Pneumonie (NSIP)	1	1/1 (100%)	
Medikamenteninduzierte Lungenschädigung	2	2/2 (100%)	
Hypersensitivitätspneumonie	7	6/7 (86%)	95%
Interstitielle Pneumonie bei Sklerodermie	2	1/2 (50%)	
Histiozytose	2	1/2 (50%)	
pANCA-positive Vaskulitis	1	0/1 (0%)	
Idiopathische Lungenfibrose (UIP, IPF)	13	9/13 (69%)	34%

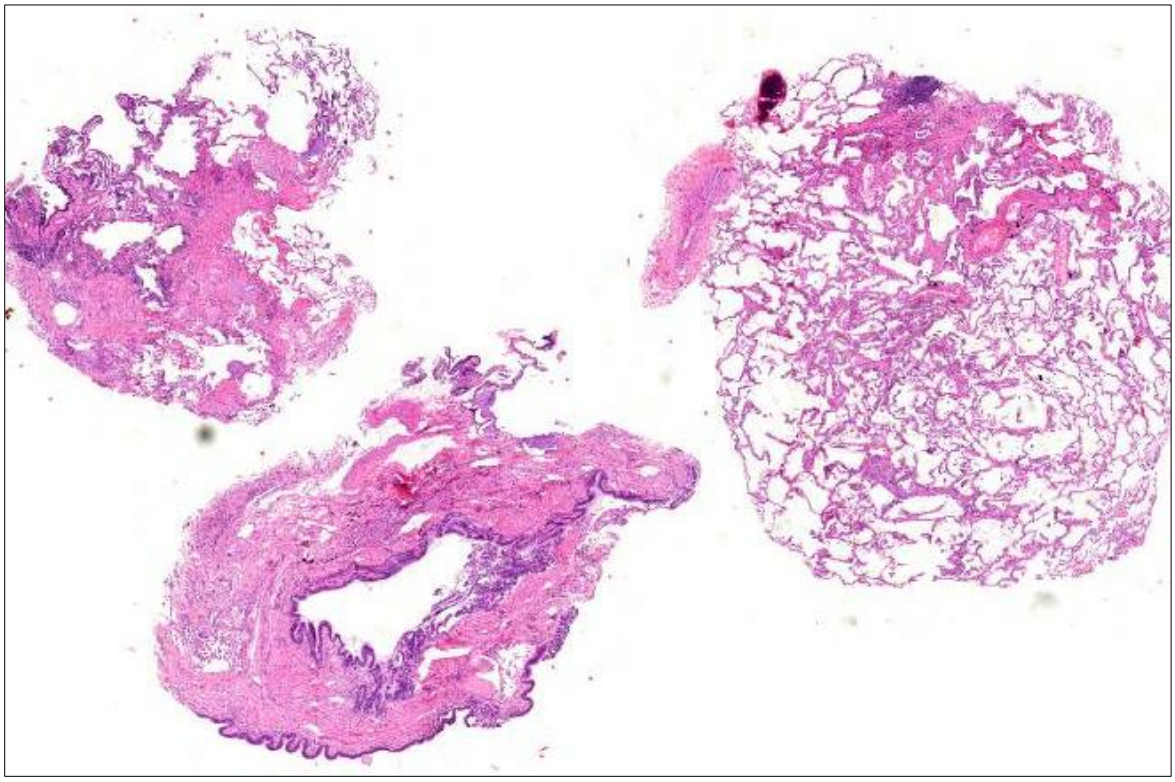

Abb.2: Schwerer Lungenumbau mit Fibrose und Fibroblastenfocus bei UIP

Arbeit 4

In dieser Arbeit wurden die Ergebnisse der Biopsien der parietalen Pleura mittels 3 unterschiedlichen Methoden verglichen: Zum einen mittels einer traditionellen starren Biopsie, zum anderen mit einer flexiblen Biopsie und letztendlich mit einer Kryobiopsie, die bislang noch keine Verbreiterung in der Diagnostik der pleuralen Läsionen gefunden hat.

Hierbei handelte es sich um ein Kollektiv aus 80 Patienten mit folgenden klinischen und histopathologischen Diagnosen:

Histopathologische Diagnosen	**Anzahl der Fälle (%)**
Idiopathische chronische Pleuritis	33/80 (41%)
Nicht kleinzelliges Lungenkarzinom	19/80 (24%)
Pleurale Karzinose bei Mammakarzinom	11/80 (14%)
Lymphom	4/80 (5%)
Pleurale Karzinose bei anderen Malignomen	4/80 (5%)
Malignes Mesotheliom	3/80 (4%)
Pleuritis tuberculosa	3/80 (4%)
Kleinzelliges Lungenkarzinom	2/80 (3%)
Asbestose	1/80 (1%)
Fälle Gesamt	80/80 (100%)

Die Frage der diagnostischen Qualität der Biopsie der Pleura parietalis stellte bei dieser Arbeit eine besondere Herausforderung dar. Es wurde entschieden, als Qualitätsmerkmale der Biopsie das Vorhandensein des subpleuralen Fettgewebes anzusehen, da dieser Faktor bei Diagnose des Pleuramesothelioms eine besondere Rolle spielt und die Infiltration des Tumors ins Fettgewebe als ein eindeutiger Beweis der Malignität anerkannt ist. In diesem Sinne stellte sich die Anzahl der Biopsien hinsichtlich der Entnahmemethode folgendermaßen dar:

	Anzahl der Biopsien	**Miterfasstes Fettgewebe**	**Kein Fettgewebe miterfasst**	**Prozentsatz der Biopsien mit Fettgewebe**
Starre Biopsie	205	129	76	62.9
Flexible Biopsie	104	41	63	39.4
Kryobiopsie	99	49	50	49.5
Insgesamt	408	219	189	53.7

In 79/80 der Fälle (98.7%) wurde die Diagnose durch eine rigide Biopsie gestellt. Die Diagnose fand sich in 73 von 80% der Fälle (91.3%, 95%CI 86.0 – 96.5%) zu Kryobiopsien und in 74 von 80 Fällen (92.5%, 95%CI 88.6 – 97.4%) für flexible Zangenbiopsien wieder. Somit war die diagnostische Ausbeute der Kryobiopsien schlechter als bei starrer Thorakoskopietechnik (12.7%), aber nicht signifikant schlechter als bei flexibler Zangenbiopsie (6.5%). Bei 73 Patienten (91%) wurde durch alle 3 Methoden die gleiche Diagnose gestellt, während in 3 von 80 Fällen (4%) eine Diagnosestellung nur durch eine rigide Biopsie möglich war.

In einem Fall bei einer durch alle 3 Methoden diagnostizierten idiopathischen Pleuritis wurde im 12-monatigen Verlauf die Diagnose eines malignen Mesothelioms gestellt und die ursprüngliche Diagnose somit klinisch und später histopathologisch revidiert, ohne dass in den ursprünglichen Proben auch bei einer erneuten Begutachtung die Diagnose eines Mesothelioms möglich war.

Arbeit 5

Hierbei handelt es sich um einen Fallbericht einer Patientin mit zahlreichen kleinen nodulären Formationen in beiden Lungen und klinischem Verdacht auf Sarkoidose. Mittels Kryobiopsie wurden mehrere Proliferate von neuroendokrinen Zellen diagnostiziert, die, insbesondere in Zusammenhang mit radiologischen Befunden, einer diffusen idiopathischen neuroendokrinen Hyperplasie der Lunge entsprechen. Mögliche Differenzialdiagnosen waren andere noduläre Lungenerkrankungen, in 1. Linie Sarkoidose.

DISKUSSION

In den vorliegenden 5 Arbeiten wurde versucht, unterschiedliche Aspekte der Kryobiopsie als Untersuchungsmethode zu beleuchten.

Zunächst wurden die morphometrischen Effekte der Methode auf die gewonnenen Gewebeproben untersucht. Es wurde deutlich gezeigt, dass die durch Kryobiopsie gewonnenen Proben größer und aufgrund von fehlenden Artefakten leichter zu beurteilen sind, wobei dieser letzte Aspekt als solcher für die Diagnostik in keiner weiteren Arbeit speziell untersucht wurde. Weiterhin zeigte sich ein deutlicher Zugewinn der Proben hinsichtlich des Anteils des Lungenparenchyms, was speziell in der Diagnostik der interstitiellen Erkrankungen von entscheidender Bedeutung ist. Wie bereits oben erwähnt,

wurde in der Literatur bei den zumindest theoretischen Überlegungen zur Repräsentativität einer peripheren Lungenbiopsie eine Mindestanzahl von 20 Alveolen pro Probe vorgeschlagen (17). Anzumerken ist, dass bei interstitiellen Lungenerkrankungen die diagnostische Ausbeute der konventionellen Zangenbiopsie offenbar von der Erkrankung abhängig ist. Während manche Autoren versuchen, diese Ausbeute nach Entität zu differenzieren, ist in dem entsprechenden Kapitel des klassischen Nachschlagewerks von Katzenstein (19) eine Differenzierung zwischen immunsupprimierten und nicht immunsupprimierten Patienten zu finden. Offenbar hat beides eine Existenzberechtigung, wenn man bedenkt, dass die meisten interstitiellen Lungenerkrankungen bei immunsupprimierten Patienten diffuser Natur sind und offenbar relativ wenige Alveolen zumindest theoretisch ausreichen sollten, um die richtige Diagnose zu stellen. In diesem Sinne stellt die UIP für eine traditionelle periphere Lungenbiopsie eine besondere Herausforderung dar, da bekanntlich die meisten Veränderungen nicht peribronchial liegen (30,31) und offenbar eine gewisse Probengröße bzw. speziell die Größe des alveolären Gewebes notwendig ist, um die entscheidenden Veränderungen vom Bronchus aus zu „erreichen“. So muss man davon ausgehen, dass bei einer idiopathischen Lungenfibrose die Veränderungen in den meisten Fällen nicht erfasst werden können. In einer Arbeit von Barbescu (15) wurden die bronchialen Zangenbiopsien bei radiologisch definierten UIP-Fällen retrospektiv unter Kenntnis der Diagnose nochmals inspiziert, um eine histologische Sicherung dieser Diagnose herbeizuführen. In 7 von 21 Fällen wurden Fibroblastenfoci und eine sogenannte pachtwork-Fibrose beobachtet. Die Autoren empfahlen jedoch nicht, die transbronchiale Biopsie als Standard-Untersuchungsmethode speziell bei UIP zu verwenden und verwiesen darauf, dass es sich bei den retrospektiv untersuchten Fällen um klare radiologische und klinische Befunde handelte. Insgesamt erfolgte ein Paradigmenwechsel bei der Einschätzung der peribronchialen Lage der fibrotischen Veränderungen in Präparaten der offenen Lungenbiopsie. Diese wurden als „airway–centered interstitial fibrosis“ definiert und werden z.Z. als Erscheinungsform einer chronischen Hypersensitivitätspneumonie angesehen (32), auch wenn Fibroblastenfoci nachweisbar und Granulome oder Cholesterinkristallspalten nicht vorhanden sind (30,33). Wie unsere Ergebnisse zeigen, ist dieses Problem durch die Kryobiopsie zumindest in einem großen Teil der Fälle lösbar. Da die für eine Hypersensitivitätspneumonie entscheidenden granulomatösen Veränderungen mit Riesenzellen und Cholesterinkristallspalten für gewöhnlich peribronchial liegen, kann man

davon ausgehen, dass auch diese durch eine Kryobiopsie miterfasst werden sollten. Voraussetzung dafür wäre zumindest theoretisch das Vorhandensein des alveolären Parenchyms. Bei den typischen UIP-Veränderungen in der Kryobiopsie wäre jedoch eine Diskussion mit radiologisch tätigen Kollegen zu erwägen. Auch wenn die Gewebsstücke der Kryobiopsie deutlich größer sind als die der transbronchialen Biopsie, so sind sie jedoch wahrscheinlich nicht mit denen der offenen Lungenbiopsie gleichzustellen. So wird es im Einzelfall schwer zu entscheiden sein, was in einer Kryobiosie das peribronchiale Kompartiment repräsentiert. Grundsätzlich erscheint es ratsam, sich bei typischen histologischen UIP-Veränderungen zu vergewissern, dass radiologisch tatsächlich auch ein konsistentes Muster vorliegt. Diese Überlegung beruht auf der Tatsache, dass die Hypersensitivitätspneumonien, auch in ihrer chronischen Form, ein anderes radiologisches Muster zeigen, wie z.B. Fehlen von einem kraniokaudalen Gradienten und Milchglasveränderungen (34).

Bei der nichtspezifischen interstitiellen Pneumonie (NSIP) darf man generell gute Ergebnisse erwarten, wobei dies bei nur einem Fall in der Arbeit 3 sowie sehr kleinen Zahlen für diese Entität in der Literatur nur eine Vermutung ist (35,36,37). Auch in der eigenen Serie in der vorgelegten Arbeit 3 war diese Entität nur mit einem Fall repräsentiert.

Insgesamt erspart die diagnostisch erfolgreiche Kryobiopsie dem Patienten eine offene Lungenbiopsie (35, 36, 37), was speziell für Fälle mit idiopathischer Lungenfibrose vom größten Interesse ist, da diese aufgrund ihres Allgemeinzustandes und bei in der Regel fortgeschrittenem Alter bei dieser Erkrankung nicht operationsfähig sind. Auch ist speziell bei UIP/IPF das Risiko einer Exacerbation ebenfalls präsent, obwohl die statistischen Daten unterschiedlich sind (38, 39, 40, 41), speziell für die IPF werden bei einzelnen Untersuchungen auch Zahlen bis zu 20% angegeben. In einer großen Studie (42) ergab sich beispielsweise eine postoperative Sterberate bei IPF-Patienten von 5,1% und für die Patienten mit einer rheumatisch-assoziierten Lungenerkrankung von 6%.

Im Allgemeinen bedeutet der Einsatz der Kryobiopsie offenbar einen logistischen Vorteil innerhalb einer Klinik, da die Patienten für eine offene Lungenbiopsie nicht verlegt werden müssen, somit werden offenbar auch Kosten eingespart. Bei guter Organisation innerhalb einer Klinik und bei Notwendigkeit der ohnehin wünschenswerten bronchoalveolären Lavage (BAL) ist es vorstellbar, sowohl die BAL als auch die Kryobiopsie in derselben Sitzung

durchzuführen. In einem systematischen Review von Sharp et al wird auf der Basis der Analyse der zahlreichen Studien (darunter auch die hier vorgelegte Arbeit 4) bei einer kumulativen/statistischen diagnostischen Ausbeute von 64% und Pneumothoraxrisiko von 10% sowie Blutungsrisiko von 21% (in unserem Kollektiv in der Arbeit 4 sind keine aufgetreten) ein Kostenersparnis pro Patienten von 210 Pfund im 1. Jahr und 647 Pfund für das 2. Jahr (für das englische Gesundheitssystem) errechnet (43).

Bei den Tumorpatienten (Arbeit 2) ergibt sich durch das Einsetzen der Kryobiopsie ein diagnostischer Vorteil sowohl bei zentralen als auch bei peripheren Tumoren. Leider war es in unserer Arbeit nicht möglich, zwischen den exophytischen und submukösen endoskopischen Befunden zu unterschieden, sodass die eigentliche Zielgruppe (zumindest theoretisch) der Kryobiopsie als Methode nicht gut genug charakterisiert wurde. Eine solche Analyse wurde im Rahmen einer multizentrischen Studie vorgenommen, die gleichzeitig mit der vorgelegten Arbeit durchgeführt wurde (44). Für eine verlässliche Korrelation zwischen den einzelnen Entitäten und der Kryobiopsie waren die Fallzahlen nicht ausreichend. Es ist jedoch zu vermuten, dass bei Adenokarzinomen analog zu den submukösen Tumoren der Vorteil der Kryobiopsie größer sein dürfte, da hier die Bronchusschleimhaut als solche zumindest nicht immer befallen ist und die eigentliche Erkrankung aus dem Lungenparenchym heraus entsteht. Beim Plattenepithelkarzinom hingegen ist die Bronchialschleimhaut der primäre Entstehungsort der Erkrankung, wobei hier auch wiederum der bereits erwähnte Unterschied zwischen exophytischen und submukösen Tumoren speziell bei dieser Entität vom Interesse ist.

Bei der Arbeit Nummer 4 wurde die Studie unter der Annahme durchgeführt, dass die Kryobiopsie bessere Ergebnisse als die bisher verwendeten Methoden der starren und flexiblen Biopsie zeigt. Zu erwarten war, dass die Kryobiopsie zudem größere Proben zur Verfügung stellt und somit für bessere diagnostische Sicherheit sorgt. Auf Grund unserer Daten lässt sich jedoch lediglich eine diagnostische Gleichwertigkeit der flexiblen Biopsie und der Kryobiosie feststellen. Die Kryobiopsie hätte jedoch thorakoskopisch Vorteile, da hier die Vorzüge einer flexiblen Technik mit größeren Proben und somit einer besseren Diagnostik vereint sind. Zudem zeigten die pleuralen Kryoproben keine Artefakte, dieses Phänomen wurde bereits in der Arbeit 1 beschrieben. Es zeigten sich keine Komplikationen der zu untersuchenden Methode, dies wurde vor dieser Untersuchung bereits von Rozman et al.

beschrieben (45). Einen problematischen Sonderfall stellt in diesen Zusammenhang das maligne Mesotheliom dar. Bei dieser Erkrankung kommt es immer wieder zu diagnostischen Schwierigkeiten, da als Beweis der Malignität die Infiltration des unter dem Mesothel und Bindegewebe liegenden Fettgewebes allgemein anerkannt ist (46). Das Mesotheliom mit lediglich mesenchymalen Proliferaten ohne Infiltration des Fettgewebes bzw. ohne im Präparat sichtbares Fettgewebe (Frühemesotheliom im deutschsprachigen Schrifttum (47)) wird in der Literatur diskutiert, die Einschätzung der Desmoplasie als diagnostisches Kriterium ist jedoch vermutlich schwer reproduzierbar, solche Fälle gelten insgesamt als schwer diagnostizierbar und bedürfen in der Regel Zusatzuntersuchungen einschließlich p16-in-situ-Hybridisierung (46,48). Insgesamt zeigt die Studie auch in der Pleura gute Ergebnisse, wobei das erhoffte Ziel der Gleichwertigkeit der Kryobiopsie gegenüber der starren Biopsie nicht gezeigt werden konnte. Zu bemerken ist jedoch, dass die Anzahl der Patienten mit malignem Mesotheliom in unserer Gruppe mit 3 von 80 sehr gering war, sodass das eigentliche Dilemma aus der Sicht eines Pathologen nicht gelöst ist und eine der Zielfragen nicht beantwortet wurde. Auch in einer vor kurzem erschienenen Studie (49) zeigten sich ähnliche Ergebnisse ohne signifikanten diagnostischen Unterschied zwischen 2 Methoden. In dieser Untersuchung waren von insgesamt 139 Patienten lediglich 3 Fälle mit einem primär pleuralen Tumor, sodass auch hier diese zentrale Frage nicht gelöst werden konnte. Vermutlich ist hierzu eine multizentrische Studie bzgl. dieser speziellen Entität notwendig.

In der letzten Arbeit zeigte sich im vorgelegten Fallbericht ein Ausrufungszeichen für die vorgestellte Methode in einem Fall mit mikronodulären diffusen pulmonalen Veränderungen. Die hier diagnostizierte diffuse idiopathische neuroendokrine Hyperplasie (DIPNECH) der Lunge ist in der Regel ein Zufallsbefund in Resektionspräparaten, in den meisten Fällen ohne klinische Bedeutung. In diesem speziellen Fall hatte die Diagnose nicht nur differenzialdiagnostische (zum Beispiel mit Sarkoidose), sondern auch therapeutische und prognostische Bedeutung, da die Patientin unter bronchoobstruktiven Beschwerden leidet, die möglicherweise mit der hier festgestellten Erkrankung zusammenhängen, wie in der Literatur aufgezeigt wurde (50).

Insgesamt zeigten die vorgelegten Arbeiten verschiedene Aspekte des Einsatzes der Kryobiopsie bei tumorösen und diffusen Lungenerkrankungen sowie bei histologischer Aufarbeitung der pleuralen Läsionen.

FAZIT

1. Aus morphometrischer Sicht zeigt die Kryobiopsie der Lunge deutliche Vorteile gegenüber der herkömmlichen transbronchialen Zangenbiopsie. Es wird mehr Gewebe gewonnen, dieses Gewebe enthält in der Regel mehr alveoläres Parenchym, welches weniger Artefakte zeigt.
2. Bei der Diagnostik der Lungentumoren führt offenbar alleine der Zugewinn der Gewebsmasse zu einer besseren diagnostischen Ausbeute.
3. Durch erhöhte Anzahl der Fälle mit miterfassten alveolären Parenchym steigert sich die diagnostische Ausbeute auch in den Fällen mit interstitiellen Lungenerkrankungen deutlich. Das erspart dem Patienten in der Regel eine offene Lungenbiopsie. Dies ist in 1. Linie für die Fälle mit einem sogenannten UIP-Muster interessant, die aufgrund des Alters und oft bei fortgeschritten eingeschränkter Lungenfunktion nicht mehr operationsfähig sind.
4. Bei der Kryobiopsie der Pleura parietalis konnte gezeigt werden, dass die Methode der herkömmlichen flexiblen thorakoskopischen Untersuchung nicht unterlegen ist. Ein diagnostischer Vorteil gegenüber der starren Biopsie konnte nicht gezeigt werden, wobei die Patientenanzahl möglicherweise zu klein ist bzw. eine erneute multizentrische Studie speziell zum Thema malignes Mesotheliom aufgelegt werden sollte.

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EIDESSTATTLICHE VERSICHERUNG

„Ich, Sergej Griff, versichere an Eides statt durch meine eigenhändige Unterschrift, dass ich die vorgelegte Dissertation mit dem Thema: „Histopathologische Aspekte der Kryobiopsie der Lunge (Einfluss der Art der Materialgewinnung auf die Diagnosefindung in der Lungenpathologie)“ selbstständig und ohne nicht offengelegte Hilfe Dritter verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel genutzt habe.

Alle Stellen, die wörtlich oder dem Sinne nach auf Publikationen oder Vorträgen anderer Autoren beruhen, sind als solche in korrekter Zitierung (siehe „Uniform Requirements for Manuscripts (URM)“ des ICMJE -www.icmje.org) kenntlich gemacht. Die Abschnitte zu Methodik (insbesondere praktische Arbeiten, Laborbestimmungen, statistische Aufarbeitung) und Resultaten (insbesondere Abbildungen, Graphiken und Tabellen) entsprechen den URM (s.o) und werden von mir verantwortet.

Meine Anteile an den ausgewählten Publikationen entsprechen denen, die in der untenstehenden gemeinsamen Erklärung mit dem/der Betreuer/in, angegeben sind. Sämtliche Publikationen, die aus dieser Dissertation hervorgegangen sind und bei denen ich Autor bin, entsprechen den URM (s.o) und werden von mir verantwortet.

Die Bedeutung dieser eidesstattlichen Versicherung und die strafrechtlichen Folgen einer unwahren eidesstattlichen Versicherung (§156,161 des Strafgesetzbuches) sind mir bekannt und bewusst.“

22.03.18

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Unterschrift

ANTEILSERKLÄRUNG AN DEN ERFOLGTEN PUBLIKATIONEN

Sergej Griff hatte folgenden Anteil an den folgenden Publikationen:

1. **Griff S**, Ammenwerth W, Schönfeld N, Bauer TT, Mairinger T, Blum TG, Kollmeier J, Grüning W.: Morphometrical analysis of transbronchial biopsies. Diagn Pathol. 2011; 6(53):1-6.
   Die unten aufgelisteten Punkte präzisieren den Eigenanteil, den S. Griff in oben genannter folgender Publikation geleistet hat:
   - Definition der Fragestellung hinsichtlich der morphologischen Unterschiede Kryobiopsie und konventionellen Biopsie der Lunge
   - Studiendesign
   - Morphologische Beurteilung der histologischen Proben hinsichtlich der Artefakte und des Vorhandenseins des alveolären Lungenanteils.
   - Etablierung und Durchführung Morphometrie mittels ZEISS-MIRAX Scannersystem in allen zu untersuchenden Präparaten
   - Verfassung des Manuskripts inklusive aller Tabellen und Abbildungen Tabelle 1 und 2, Abb. 1)
   - Literaturrecherche und Erstellen der Literaturliste

2. Grüning W, Ammenwerth W, Wurps H, Kollmeier J, Blum T, Schönfeld N, **Griff S**, Bauer TT: Diagnostischer Wert und Sicherheit der Bronchoskopischer Kryotechnik im Routineeinsatz bei Verdacht auf Lungenkarzinom. Pneumologie. 2013;67:676-682.
   Die unten aufgelisteten Punkte präzisieren den Eigenanteil, den S. Griff in oben genannter folgender Publikation geleistet hat:
   - Morphologische Beurteilung der Proben
   - Auswertung der Forschungsergebnisse
   - Erstellen der Tabellen 1-3 und der Abbildung 1
   - Diskussion des Manuskripts mit dem Erstautor, insbesondere der Abschnitte mit Bezug auf histologische Fragestellungen

3. **Griff S**, Schönfeld N, Ammenwerth W, Blum TG, Grah C, Bauer TT, Grüning W, Mairinger T, Wurps H.: Diagnostic yield of transbronchial cryobiopsy in non-neoplastic lung disease: a retrospective case series. BMC Pulm Med. 2014;14(171):1-6.
   Die unten aufgelisteten Punkte präzisieren den Eigenanteil, den S. Griff in oben genannter folgender Publikation geleistet hat:
   - Problemdefinition
   - Zusammenstellung des Patientenkollektivs (Tabelle 1)
   - Beurteilung der Proben hinsichtlich des morphologischen Musters und endgültiger Diagnose
   - Anpassung der veränderten diagnostischen Kriterien der interstitiellen Lungenerkrankungen in der Kryobiopsie, da die klassischen Kriterien an den Präparaten der offenen Lungenbiopsie etabliert wurden

- Statistische Auswertung der Forschungsergebnisse (Tabelle 1)
- Anfertigung der Abbildungen (Abb. 1 bis 4)
- Entwurf des Textes
- Literaturrecherche
- Studiendesign

4. Wurps H, Schönfeld N, Bauer TT, Bock M, Duve C, Sauer R, Mairinger T, **Griff S**: intra-patient comparison of parietal pleural biopsies by rigid forceps, flexible forceps and cryoprobe obtained during medical thoracoscopy: a prospective series of 80 cases with pleural effusion. BMC Pulm Med. 2016;16(98):1-7.
   Die unten aufgelisteten Punkte präzisieren den Eigenanteil, den S. Griff in oben genannter folgender Publikation geleistet hat:
   - Zusammenstellung der Forschungsgruppe
   - Festlegung des Patientenkollektivs
   - Kritische Begutachtung der histologischen Proben im Sinne des Studiendesigns
   - Morphologische Beurteilung der histologischen Proben zwecks Überprüfung der bereits gestellten Diagnosen (Tabelle 2)
   - Anfertigung der Bilder (Abb. 2 bis 5)
   - Prüfung des Forschungskonzepts
   - Prüfung der Literatur
   - Überwachung der morphometrischen Messungen
   - Auswertung der Ergebnisse mit dem Erstautor (Tabelle 2, Abb. 1)
   - Freigabe des Manuskripts für die Einreichung

5. Sauer R, **Griff S**, Blau A, Franke A, Mairinger T, Grah C.: Diffuse idiopathic pulmonary neuroendocrine cell hyperplasia diagnosed by transbronchial lung cryobiopsy: a case report. J Med Case Rep. 2017;11(95):1-4.
   Die unten aufgelisteten Punkte präzisieren den Eigenanteil, den S. Griff in oben genannter folgender Publikation geleistet hat:
   - Literaturrecherche
   - Festlegung der Diagnose unter Berücksichtigung der klinischen Daten und der Radiologie
   - Morphometrische Messungen
   - Erstentwurf des Textes.
   - Anfertigung der Bilder (Abb. 1 bis 3)

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Unterschrift, Datum und Stempel
des betreuenden Hochschullehrers/der
betreuenden Hochschullehrerin

Unterschrift des Doktoranden/
der Doktorandin

DRUCKEXEMPLARE DER AUSGEWÄHLTEN PUBLIKATIONEN

Arbeit 1

Griff S, Ammenwerth W, Schönfeld N, Bauer TT, Mairinger T, Blum TG, Kollmeier J, Grüning W: Morphometrical analysis of transbronchial cryobiopsies. Diagn Pathol. 2011;6(53):1-6.

DIAGNOSTIC PATHOLOGY

RESEARCH Open Access

Morphometrical analysis of transbronchial cryobiopsies

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Abstract

The recent introduction of bronchoscopically recovered cryobiopsy of lung tissue has opened up new possibilities in the diagnosis of neoplastic and non-neoplastic lung diseases in various aspects. Most notably the morphological diagnosis of peripheral lung biopsies promises to achieve a better yield with a high quality of specimens. To better understand this phenomenon, its diagnostic options and perspectives, this study morphometrically compares 15 cryobiopsies and 18 transbronchial forceps biopsies of peripheral lung tissue a priori without considering clinical hit ratio or integration of results in the clinical diagnostic processing. Cryotechnically harvested specimens were significantly larger (mean: 17.1 ± 10.7 mm^2 versus 3.8 ± 4.0 mm^2) and contained alveolar tissue more often. If present, the alveolar part in cryobiopsies exceeded the one of forceps biopsies. The alveolar tissue of crybiopsy specimens did not show any artefacts. Based on these results cryotechnique seems to open up new perspectives in bronchoscopic diagnosis of lung disease.

Background

The diagnostic yield of transbronchial lung biopsy (TBB) by forceps is a function of biopsy quality defined by specimen size and preservation of tissue architecture. In addition, artifacts may considerably affect the interpretation of the tissue obtained.

The tissue sample delivered in clinical routine usually consists of one or more lung pieces averaging 1 to 2 mm in size [1-4]. It is difficult to report the diagnostic accuracy of TBB, because they are taken for various indications. The majority of large case series report a diagnostic accuracy of 50% to 70% depending on the indication, size and location of the lesion [1,3,5-10]. Peripheral tumour lesions can be diagnosed in up to 57% of patients [9,11]. In diffuse lung diseases, the overall efficacy is probably lower, whereas the technique seems to be highly efficient in sarcoidosis and cryptogenic organising pneumonia. The results in usual interstitial pneumonia, pneumoconiosis or pulmonary histiocytosis X are poor [1,4,10,12]. This large variation is due to the different importance of alveolar tissue which is usually underrepresented in TBB. Moreover, information about distribution of the pathologic pattern throughout the lungs can principally not be provided by TBB.

Cryosurgical techniques have been used in the airways as early as 1968 [13]. The cryotechnique was mainly used for palliative treatment of obstructing endobronchial tumors [14-16]. Cryotechniques use very low temperatures induced by rapid expansion of gas released at high flow (Joule-Thompson effect) and leads to adhesion of the specimen to the probe. Such pieces of tissue can be extracted with the freeze-thaw cycle without increasing the danger of life threatening complications [17,18].

With the implementation of flexible probes for the diagnostic work-up of patients with endobronchial tumor lesions, cryobiopsy was introduced recently on a routine basis and found to be also safe in a routine diagnostic setting [17,19]. The biopsies obtained in these experiments were reported to be larger and diagnostically more valuable. An animal study supports these hypothesis by showing that the preservation of samples and sample size can be improved by cryotechnique [17,18]. Similar results have been shown from central cryobiopsies in a study on efficacy of cryobiospies in cancer patients [20].

The aim of our study was to evaluate cryotechnique for peripheral transbronchial lung biopsies with the focus on sample adequacy for diagnostic purposes with

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respect to sample size and proportion of alveolar tissue retrieved.

Methods

This is a prospective case series of 15 patients, who underwent flexible bronchoscopy including transbronchial cryobiopsies. A series of 18 patients undergoing conventional peripheral transbronchial biopsies by forceps were selected as the control group. Patient characteristics (localization, clinical indication) are listed in tables 1 and 2.

Cryobiopsies and conventional transbronchial biopsies were obtained during flexible bronchoscopy with sedation and local anaesthesia using a flexible bronchoscope (1T 160 and 1T 180, Olympus Corp. Tokyo Japan). The cryoprobe or forceps were introduced into the selected area under fluoroscopic guidance. For all samples, a distance of approximately 10-20 mm from the thoracic wall was considered optimal. Once brought into position, the probe was cooled for approximately five seconds and then retracted with the frozen lung tissue being attached on the probe's tip. The frozen specimen was thawed in saline and fixed in 4% buffered formalin. All specimens won by TBB were stored in Formalin only and the forceps size used was 1.8 mm (Boston Scientific Radial Jaw™ Natick, MA USA).

For this study a signed informed consent of all patients was obtained. The cryotechnique is a technology authorized by the German medicinal products act and was applied depending on the individual medical indication. Since there was no patient randomization and no specimens beyond the medical indication were harvested, formal approval of an ethics committee was not obtained.

Only one cryobiopsy was taken, whereas the number of conventional TBB varied from 1 to 4, depending on the investigator. All biopsies were processed conventionally by serial sectioning of at least 12 H&E stained section steps to avoid incomplete sectioning of particles. Subsequently the biopsies were rated regarding quality and quantity by two experienced lung pathologists (SG & TM), rendering a consensus about biopsy quality as well as presence and amount of artefacts (Tables 1 and 2).

Hematoxylin-eosine slides were scanned by a ZEISS-MIRAX Midi Slide scanning system using the Mirax Viewer Image Software Ver[1,6]. (Zeiss Microimaging, Oberkochen, Germany and 3DTech, Budapest, Hungary) (Figure 1). The total area of the biopsy specimens and the area of the alveolar part were measured by interactive circling of the biopsy section and its alveolar part. In each conventional TBB, all tissue samples were measured, but only the largest in size was included in the subsequent analysis (see discussion). All areas were calculated automatically and expressed in μm^2.

Statistics

All data were analyzed and processed using statistical software (Statistical Package for Social Sciences, Version 14.0; SPSS, Chicago, IL, USA) on a Windows XP operating system (Microsoft; Redmond, WA, USA). Results were expressed as frequencies or as mean ± SD unless indicated otherwise. The $\chi 2$-test was used to compare proportions, and Student t test was used to compare means. The significance level of all analyses was set to 5%, and exact p values are reported. Results were expressed using descriptive statistics.

Table 1 Measurements of the cryobiopsies

Specimen's number	Whole area [mm^2]	Alveolar part within biopsy	Area of alveolar part [mm^2]	Artifacts of alveolar part	Localisation	Clinical indication
1	31.885	No			OL right	Fibrosis
2	0.633	No			ML	Infiltration
3	4.913	Yes	1.799	None	ML	Infiltration
4	8.813	Yes	5.379	None	OL left	Infiltration
5	28.699	Yes	22.689	None	Lingula	Fibrosis
6	9.125	No			OL right	Fibrosis
7	29.353	Yes	13.303	None	OL left	CUP
8	21.299	Yes	7.035	None	OL right	Fibrosis
9	16.259	No			Ul right	Infiltration
10	14.613	Yes	13.204	None	OL right	Infiltration
11	13.815	Yes	0.449	None	ML	Infiltration
12	31.7999	Yes	25.155	None	UL left	Infiltration
13	28.202	Yes	24.979	None	ML	ILD
14	6.304	Yes	5.684	None	UL left	ILD
15	10.440	Yes	7.503	None	UL left	Infiltration

Table 2 Measurements of the forceps biopsies

Specimen's number	Whole area of the largest specimen [mm^2]	Alveolar part within biopsy	Area of alveolar part [mm^2]	Artifacts of alleolar part	Localisation	Clinical indication
1	2.333	Yes	1.058	Severe	UL right	Fibrosis
2	2.093	Yes	0.982	Mild	OL right	Fibrosis
3	1.007	No			ML	Fibrosis
4	1.384	No			ML	Fibrosis
5	4.344	Yes	1.612	Mild	UI left	ILD
6	1.714	Yes	1.105	Mild	Lingula	Infiltration
7	3.684	Yes	1.195	Moderate	OL right	Infiltration
8	5.142	Yes	2.674	Moderate	OL right	Infiltration
9	2.688	Yes	0.430	Severe	OL right	Infiltration
10	11.828	No			OL right	Peripheral lesion
11	2.424	Yes	1.511	Moderate	UL left	Peripheral lesion
12	0.572	No			ML	Infiltration
13	2.806	Yes	2.372	Severe	UL right	Fibrosis
14	1.422	No			OL right	Infiltration
15	6.817	Yes	5.972	moderate	UL right	Infiltration
16	2.043	No			UL right	Infiltration
17	15.713	No			UL right	Infiltration
18	0.366	No			Lingula	Fibrosis

Results

Morphometric data of all samples including presence of alveolar part, its size and artefacts within alveolar tissue are listed in tables 1 and 2, and representive specimens retrieved with the two methods under comparison are illustrated in Figure 2 and 3.

Cryoprobes were larger and much more representative of real lung structure featuring pathological attributes as compared to small biopsy specimens of the forceps technique (Figure 1). Specimen size in cryobiopsies was significantly larger than in those obtained by forceps (mean: 17.1 ± 10.7 μm^2 versus 3.8 ± 4.0 μm^2, n = 15 and 18 respectively; $p < 0.001$) (Figure 2). In cryobiopsies, alveolar tissue was found in 11 of 15 (73%), in forceps biopsies only in 10 of 18 (56%), thus showing a trend for cryobiopsy containing alveolar tissue more

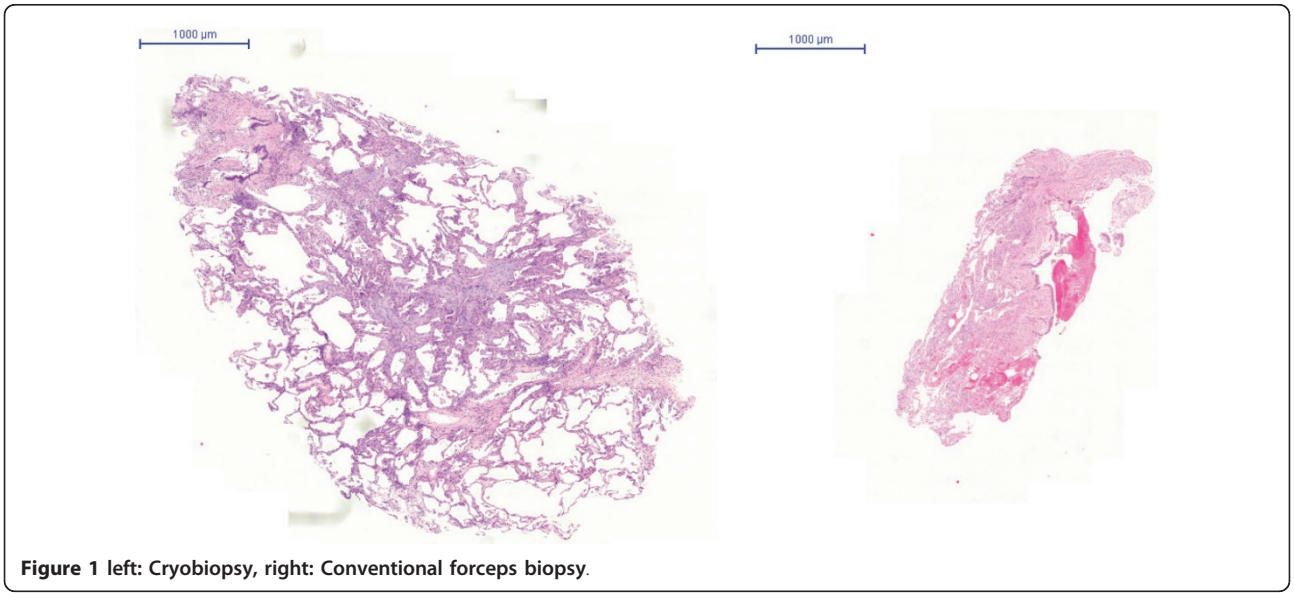
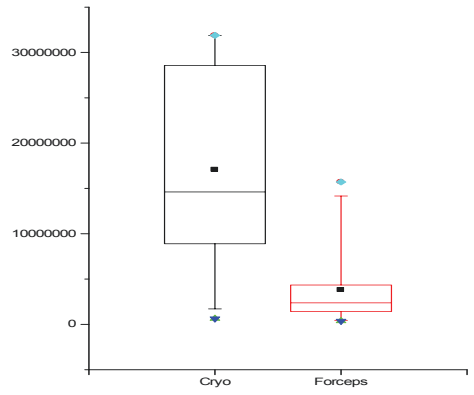


Figure 1 left: Cryobiopsy, right: Conventional forceps biopsy.

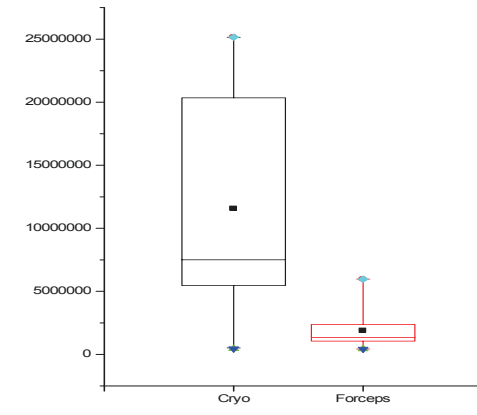


	Mean	MAX	Min	P1	p99	sd
Cryo	17.0768	31.885	0.633	0.633	31.885	10.66
Forceps	3.799	15.713	0.366	0.366	15.713	4.027

Figure 2 Specimen Size, graph in μm², statistical data in mm².

often (p = 0.290). The specimens lacking alveolar tissue either contained only bronchial mucosa and cartilage or proved to be flat long bands of inner bronchial wall lining, the latter phenomenon exclusively seen in cryoprobes. In specimens with alveolar tissue, its size was significantly larger in the cryobiopsies (11.6 ± 9.1 μm^2 versus 1.9 ± 1.6 μm^2 in the forceps group, n = 11 and 10 respectively; p = 0.004) (Figure 3). When the pathologists rated the presence of artefacts within the alveolar tissues, none of the specimens taken by cryobiopsy technique (0/11; 0%) showed parenchymal damage due to compression, whereas all (10/10; 100%; p = 0.005) forceps samples demonstrated at least mild parenchymal changes.

In both groups of patients there were no complication in terms of pneumothorax. In one patient of the forceps biopsy group a major bleeding (> 3 min) occurred without need of further intervention.



	Mean	MAX	Min	P1	p99	sd
Cryo	11.562	25.155	0.449	0.449	25.155	9.071
Forceps	1.891	5.972	0.430	0.430	5.972	1.580

Figure 3 Size of the alveolar part, graph in μm², statistical data in mm².

Discussion

Transbronchial forceps biopsy is a well accepted and established retrieval tool for the histologic analysis of parenchymal lung disease [1]. The requirement of histologically proven diagnosis in suspected lung cancer is evident. However, on the background of tumor heterogeneity, forceps biopsies are always hampered by small sample sizes and thus uncertain representation for the tumor. In addition, larger samples will likely become even more important regarding the steadily increasing amount of information that is expected to be gathered from tissue samples in pinhead size. Particularly surface and receptor analyses, prognostic or predictive genetic markers and/or upcoming epigenetic changes will become more important.

In non-neoplastic diseases a number of entities like sarcoidosis or bronchiolitis obliterans may be diagnosed on material obtained by transbronchial forceps biopsy. Thus far, the efficacy of TBB in non-neoplastic lung diseases has only been addressed in a few studies [1,4,10,12].

Small size is the major factor limiting the usefulness of TBB in clinical practice. Therefore the adequacy of samples has always been a matter of debate. In needle biopsies of solid organs such as kidney or liver with histologically distinct and thus countable structures (e.g., number of glomeruli), specimen adequacy can be determined easily. In contrast, specimen adequacy in lung biopsy has not yet been clearly defined and depends on clinical context. However no such approach has been implemented, because alveoli vary largely in size, shape and quality and cannot be easily rated. Moreover, two separate histologic structures - bronchioli and alveoli - should be present in a biopsy. Additionally there are several artifacts, which are typical for a forceps biopsy such as atelectasis, intraalveolar hemorrhage and so called bubble artifacts [4]. The frequent presence of artificial atelectasis may obscure diagnostic features and also be misinterpreted as interstitial fibrosis [10]. Several studies have rated the adequacy of the transbronchial biopsy based on alveolar content and specimen size. In a multivariate analysis, the number of alveolar spaces necessary for an adequate biopsy was defined as 20 [6]. Morphometry has been shown to be an efficient method to evaluate sample size [6].

A promising approach to obtain more representative samples in bronchial biopsy seems to be the introduction of modern cryoprobes. With this technique, the sample is collected while still being frozen, with the tissue attached on the frozen probe's tip. The value of the biopsy under diagnostic aspects is influenced not only by the size itself but by the absolute as well as relative content of alveolar structures, bronchial wall and neoplastic or reactive changes of the tissue samples [1,2,6,7,9,12,18].

In our series the size of the biopsy specimens differed significantly between cryoprobes and TBB specimens. However it has to be emphasized that in each TBB only one of the up to 4 biopsies was included in the analysis. Usually we decided to include the largest piece. In cases lacking alveolar tissue, present in other biopsies of the case, we included the largest alveoli-bearing biopsy specimen. The rationale behind this was the fact that for the diagnostic value of a biopsy only continuous tissue areas can be included in the diagnostic process, as topographic information is essential in histopathology, especially in diagnoses predominantly driven by pattern information. Strictly speaking every specimen in TBB has to be interpreted for its own, as the exact topographic relation between specimens of different biopsy site remains unclear, at least for the histopathologist.

Our results show an important difference between forceps biopsy and cryobiopsy. The latter samples are larger, and a trend to superiority with respect to the alveolar tissue fraction is observable. In the group of cryobiopsies we found alveolar tissue in a higher proportion of cases as compared to the group of forceps biopsies (73 vs 56%). The absolute value as well as the relative amount of alveoli is undoubtedly superior in the cryoprobe. In addition, artifacts in the alveolar part were not observed in the cryogroup but in each sample obtained by forceps biopsy.

The increased amount of tissue available for histological and molecular access may significantly improve the diagnostic value of bronchoscopical lung biopsies which has to be investigated in further studies.

Conclusions

Major findings of this study were: 1. Cryobiopsy specimens are significantly larger than those obtained by forceps transbronchial biopsies. 2. There was a tendency for alveolar tissue to be recovered more likely using cryobiopsies. 3. The size of the alveolar part in cryobiopsy specimens was significantly larger compared to specimens obtained by forceps. 4. Cryobiopsies appear of higher quality to the pathologist lacking more or less any artifacts in the alveolar parts of specimens. Cryotechnique is an important new tool for the bronchoscopic diagnosis of lung disease. The method is superior to TBB with a forceps in terms of sample size and quality. Whether this technique can replace even open surgical biopsy for the diagnosis of parenchymal lung disease needs to be addressed by prospective studies.

List of abbreviations

TBB: Transbronchal biopsy; OL: Lung upper lobe; ML: Lung middle lobe; UL: Lung lower lobe; ILD: Interstitial lung disease

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Authors' contributions

WA, NS, WG, JK collected and registered all the samples, SG and TM performed histological examinations and measurements on samples, statistical analysis was performed by TTB and TM, patient and clinical data documentation was done by TB, JK and WG, endoscopical implementation and processing of cryotechnique: WG and NS, manuscript: SG and WG, study design: TM and WG, study coordination: TM and WG.

Competing interests

The authors declare that they have no competing interests.

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Erratum zur Arbeit 1

Griff S, Ammenwerth W, Schönfeld N, Bauer TT, Mairinger T, Blum TG, Kollmeier J, Grüning W: Erratum to: Morphometrical analysis of transbronchial cryobiopsies. Diagn Pathol. 2016;11(64).

Griff et al. Diagnostic Pathology (2016) 11:64
DOI 10.1186/s13000-016-0515-1

Diagnostic Pathology

ERRATUM

Open Access

Erratum to: Morphometrical analysis of transbronchial cryobiopsies

Sergej Griff[1*], Wim Ammenwerth[2], Nicolas Schönfeld[2], Torsten T. Bauer[2], Thomas Mairinger[1], Torsten-Gerriet Blum[2], Jens Kollmeier[2] and Wolfram Grüning[2]

Erratum

The original version of this article [1] was unfortunately missing an acknowledgments section.

The information missing from this section should have noted that this work is a part of the doctoral thesis of main author Sergej Griff, being performed at the Charité, University of Medicine in Berlin, Germany.

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Reference

1. Griff S, et al. Diagnostic Pathology 2011, 6:53 DOI: 10.1186/1746-1596-6-53. http://www.diagnosticpathology.org/content/6/1/53.

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Arbeit 2

Grüning W, Ammenwerth W, Wurps H, Kollmeier J, Blum TG, Schönfeld N, **Griff S**, Bauer TT: Diagnostischer Wert und Sicherheit der bronchoskopischen Kryotechnik im Routineeinsatz bei Verdacht auf Lungenkarzinom. Pneumologie 2013; 67(12): 676-682

Arbeit 2 (Seite 34 bis einschließlich 40) darf aus rechtlichen Gründen nicht in der elektronischen Publikation veröffentlicht werden.

Zugriff unter doi:10.1055/s-0033-1344853.

Arbeit 3

Griff S, Schönfeld N, Ammenwerth W, Blum TG, Grah C, Bauer TT, Grüning W, Maringer T, Wurps H: Diagnostic yield of transbronchial cryobiopsy in non-neoplastic lung disease: a retrospective case series. BMC Pulm Med. 2014;14(171):1-6.

BMC Pulmonary Medicine

RESEARCH ARTICLE Open Access

Diagnostic yield of transbronchial cryobiopsy in non-neoplastic lung disease: a retrospective case series

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Abstract

Background: Due to the small amount of alveolar tissue in transbronchial biopsy (TBB) by forceps, the diagnosis of diffuse, parenchymal lung diseases (DPLD) is inherently problematic, with an overall low yield. The use of cryotechnique in bronchoscopy, including TBB by cryoprobe, has revealed new opportunities in the endoscopical diagnosis of malignant and non-malignant lung diseases.

Methods: To evaluate TBB by cryotechnique for non-neoplastic lung diseases, we analyzed 52 patients (mean age 63 ± 13 years) with unclear DPLD. These individuals underwent bronchoscopy with TBB by cryoprobe. Thereafter histopathological results were compared with the clinically evaluated diagnosis.

Results: No major complications were seen. Mean specimen diameter in the histological biopsies was 6.9 ± 4.4 mm (Range 2 – 22 mm). A correlation between clinical and histopathological diagnoses was found in 79% of cases (41/52). In the case of UIP (usual interstitial pneumonia) pattern, the concordance was 10/15 (66%).

Conclusion: Based on these results TBB by cryotechnique would appear to be a safe and useful method that reveals new perspectives for the endoscopical diagnosis of DPLD.

Keywords: Diffuse parenchymal lung disease (DPLD), Bronchoscopy, Transbronchial biopsy (TBB), Cryotechnique, Histopathology, Usual interstitial pneumonia (UIP)

Background

Concerning transbronchial lung biopsy (TBB) by forceps, histopathological results depend on specimen size and quality, artificial changes due to the procedure itself, and the amount of alveolar tissue contained in the sample. TBB by forceps typically delivers one or more 1–2 mm sized specimen, often with underrepresentation of alveolar tissue [1-4].

Due to the large variation of indications, as well as different sizes and locations of pulmonary lesions, diagnostic yield of TBB by forceps is severe to describe. Most recent case series specify a diagnostic accuracy of about 50% to 70% [1,3,5-11].

Diffuse parenchymal lung diseases (DPLD) are harder to diagnose by TBB with an overall lower yield. Efficacy variations depend on the underlying disease: Sarcoidosis and cryptogenic organizing pneumonia (COP) render fairly good results, whereas usual interstitial pneumonia (UIP), pneumoconiosis, respiratory bronchiolitis associated interstitial lung disease (RB-ILD), non specific interstitial pneumonia (NSIP) and pulmonary histiocytosis X show poor results [1,4,10,12,13]. This broad range is explained by the diverging importance of alveolar tissue for the histopathological diagnosis.

Due to the small yield of alveoli in TBB by forceps it is not possible to gather further information concerning the histopathological pattern of affected tissue throughout the lung. This problem is well known and has recently been discussed in literature [1,3,4,6,13-17]. Some papers do show an increase of diagnostic yield depending on the size of the specimen [6,14], however

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artificial changes due to TBB by forceps are an important limiting criterion for the pathological diagnosis of DPLD.

Cryobiopsy as a tool in bronchology has been introduced on a routine basis in recent years and has been found to be safe in a routine diagnostic setting [18,19]. Specimen size has been reported to be larger and diagnostically more valuable due to more alveolar tissue and less artificial changes. In a previous paper morphometrical benefits of cryobioptically obtained lung tissue specimen were shown and perspectives of this method in a daily routine were discussed [20]. There is evidence that cryobiopsies increase efficacy concerning histopathological tumor diagnostics in central malignant lesions [19,21].

The aim of this study was to evaluate TBB by cryotechnique for non-neoplastic diseases. Our focus was sample adequacy for diagnostic purposes, sample size and proportion of alveolar tissue retrieved, as well as the possibility of histopathological diagnosis of DPLD by cryobiopsy in correlation to clinical diagnoses.

Methods

This is a retrospective case series (June 2009 – December 2011) of 52 patients with diffuse, interstitial, non-neoplastic lung diseases who underwent flexible, fiber-optic bronchoscopy with transbronchial cryobiopsy. Additionally, all patients had routine diagnostics including lung function evaluation, chest x-ray and thoracic computed tomography (CT-scan).

For the transbronchial cryobiopsy a flexible cryoprobe with a diameter of 1.9 mm was used (flexible Kryosonde diameter 1.9 mm length 900 mm, Erbe Elektromedizin GmbH, Tübingen, Germany), the probe was cooled to a temperature of about − 77°C by carbon dioxide. The flexible bronchoscopy (1 T 160 and 1 T 180, Olympus Corp. Tokyo Japan) was performed under sedation with disoprivan or midazolam and local anaesthesia with lidocaine. The cryoprobe was introduced into the selected area with a distance of approximately 1–2 cm from the thoracic wall under radiological guidance. In this position the cryoprobe was cooled for three to five seconds and then retracted with the attached frozen lung tissue. For each patient one to two specimen were taken and then fixed in 4% buffered formalin.

To avoid incomplete sectioning of specimen particles all biopsies were conventionally processed by serial sectioning of at least 12 H & E stained section steps. Concerning quantity, quality (number of artefacts), and the amount of alveolar tissue, the biopsies were rated by two experienced lung pathologists (SG & TM).

Mirax Viewer Image Software Ver (1, 6) was used for scanning the Hematoxylin-eosine slides by a ZEISS-MIRAX Midi Slide scanning system (Zeiss Microimaging, Oberkochen, Germany and 3DTech, Budapest, Hungary). The total diameter of the biopsy specimens were measured and expressed in μm.

Histopathological changes were rated according to criteria for UIP diagnosis based on the Official ATS/ERS/JRS/ALAT Statement (22). The histological diagnosis of other entities was made by the use of classical criteria for interstial lung diseases (4).

Histopathological results and radiographic images were compared to the patients' medical history, physical examination and data of pulmonary lung function testing. At last, in an interdisciplinary setting (pathologist, radiologist, pneumologist) a diagnosis was found. Furthermore, complications seen during bronchoscopy were rated.

Statistically, results were expressed as frequencies or as mean ± SD. Chi-square-test was used to compare proportions. The significance level of the analyses was set to 5%, and exact p values were reported. Results were expressed using descriptive statistics.

Statistical software (Statistical Package for Social Sciences, Version 14.0; SPSS, Chicago, IL, USA) was used to analyze and process the data on a Windows XP operating system (Microsoft; Redmond, WA, USA).

A waiver for this study was received by the ethics committee of the Charité, Berlin, Germany ("Ethikkommission, Ethikausschuss 1 am Campus Charité – Mitte") on January 23, 2014.

Results

Overall, 52 patients with a median age of 63 ± 13 years were analyzed. 36/52 (69%) patients were male, 16/52 (31%) were female. In 41/52 cases (79%) a correlation with clinical and histopathological diagnosis was found. In 11/52 cases (21%) no match could be achieved.

Mean specimen diameter in the histological biopsies was 6.9 ± 4.4 mm (Range 2 – 22 mm). In the specimen, alveolar tissue was found in 48/52 (92%) cases. In 4/52 (8%) cases no alveolar tissue was found. In one of these four cases no histopathological diagnosis could be matched to the clinical diagnosis due to the lack of alveolar tissue. In the other three cases the diagnosis was sarcoidosis, and typical granulomas were found in the bronchial mucosa. The specimens lacking alveolar tissue either contained only bronchial mucosa and sometimes cartilage, or presented themselves as long flat bands of inner bronchial wall lining.

No major complications (pneumothorax, major bleeding >3 minutes) with need of further intervention were reported.

Table 1 shows the list of clinically diagnosed lung diseases, the number of matching histopathological findings and the average diagnostic yield of TBB by forceps reported in literature.

The HR-CT images of patients who had the clincal diagnosis of idiopathical lung fibrosis (IPF) or pulmonary

Table 1 Comparison of clinically diagnosed DPLD (with number of cases and matching histopathological findings) and averagely reported diagnostic yield by forceps biopsy

Clinical diagnosis	*Number of cases*	*Matching histopathological findings*	*Average reported diagnostic yield by forceps biopsy*
COP	9	8/9 (89%)	65% (10, 27, 28)
Rheumatoid lung disease	2	2/2 (100%)	
Sarcoidosis	12	10/12 (83%)	69% (10, 28, 29)
Alveolar microlithiasis	1	1/1 (100%)	-
NSIP	1	1/1 (100%)	-
medically-induced lung damages	2	2/2 (100%)	-
HP	7	6/7 (86%)	95% (10)
Pulmonary manifestation of scleroderma	2	1/2 (50%)	-
Histiocytosis	2	1/2 (50%)	-
pANCA-pos. Vasculitis	1	0/1 (0%)	-
IPF	13	9/13 (69%)	34% (1, 10)

manifestation of scleroderma were rated apropos of the radiological criteria for UIP after ATS (American Thoracic Society) and ERS (European Respiratory Society) [22,23]. Of these fifteen cases, fourteen (93%) showed possible or probable UIP pattern and one (7%) was inconsistent with UIP pattern.

Discussion

Cryobiopsy in our series proved to be a sufficient tool in the diagnostic processing for various diffuse, parenchymal lung diseases (DPLD). Specifically, the highest diagnostic yields were achieved in patients with sarcoidosis (83%), COP (89%), and hypersensitivity pneumonia (HP, 86%). Comparable results with the use of transbronchial forceps biopsy have been reported in recent years [1,3,5-10] (see Table 1). These good results are probably due to the location of granulomatous or other characteristic changes close to or within the bronchial wall.

Nevertheless clear distinctions became obvious between diseases that required the recognition of a gross histological pattern (UIP, NSIP, RB-ILD) and all others.

It has generally been assumed that transbronchial lung biopsies cannot be used for the diagnosis of UIP [10]. A hallmark characteristic of UIP is the patchy involvement of lung tissue, so that areas of involved parenchyma and unaffected alveoli stand next to each other. Furthermore, UIP is characterized histologically by fibrosis and chronic inflammation, i.e. features that are usual unspecific findings located in the peribronchial tissue.

In a study by Berbescu et al. [1], 22 patients with UIP pattern assessed by open lung biopsy were retrospectively analyzed concerning a pre-op achieved TBB. This revealed a characteristic histopathological UIP pattern in nine cases. Berbescu et al. concluded [1] that certain characteristic features of UIP, such as the patchwork pattern of involvement by fibrosis and temporal variability with fibroblast foci, collagen, and honeycomb changes, previously thought to be recognizable only on surgical lung biopsy specimen, can sometimes be seen on TBB specimen. The patchwork pattern is typically characterized by normal alveoli in close relation to areas of interstitial fibrosis. Its presence helps to distinguish the changes to nonspecific peribronchial fibrosis where there is a gradual transition from normal to abnormal.

In our series of transbronchial cryobiopsies an UIP pattern was diagnosed in two-thirds (10/15, 67%) of the

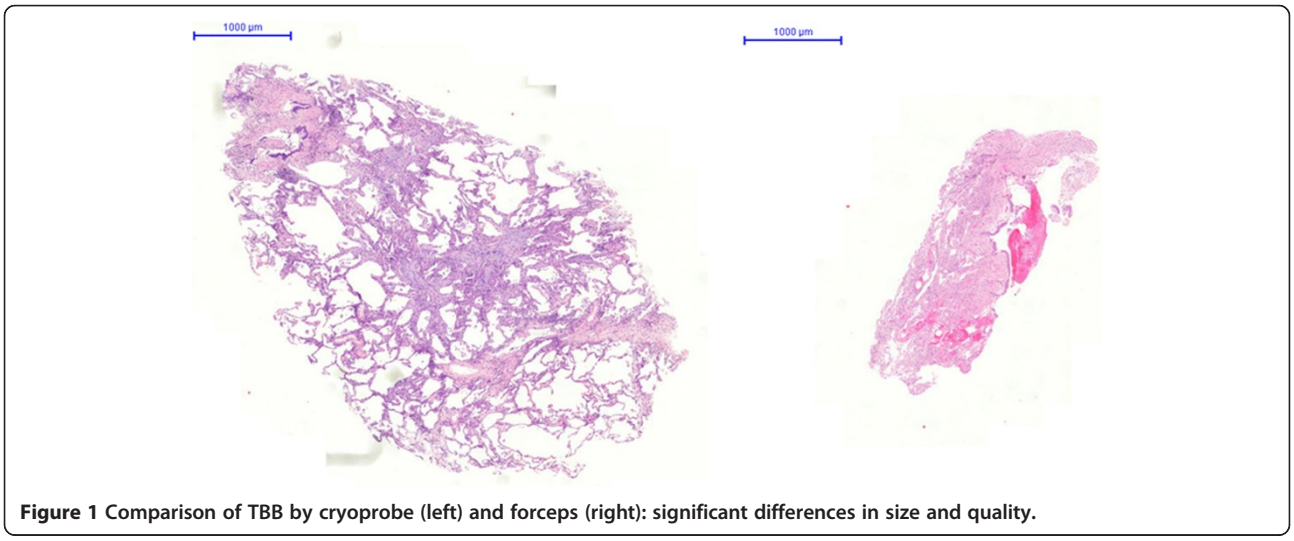

Figure 1 Comparison of TBB by cryoprobe (left) and forceps (right): significant differences in size and quality.

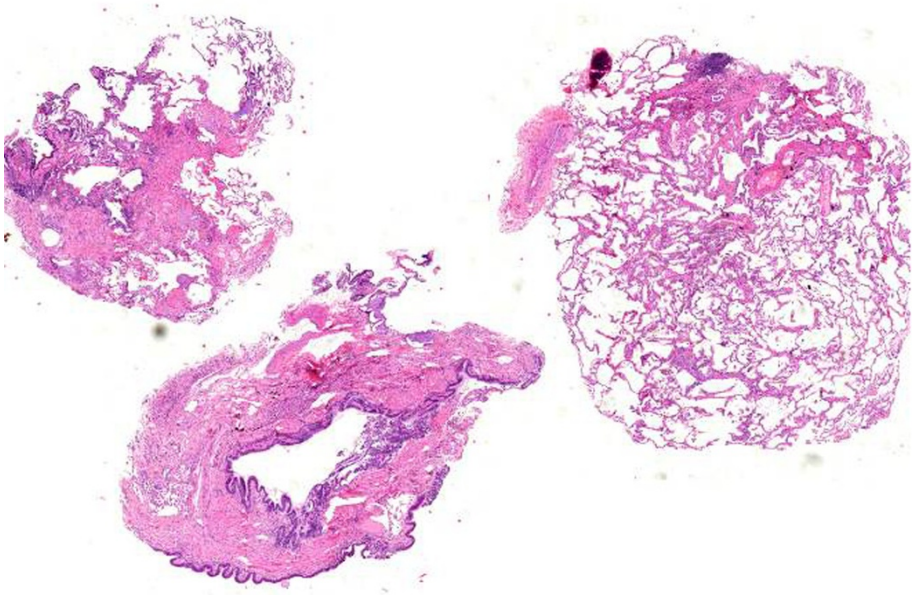

Figure 2 Patient with radiological UIP pattern. Overview of transbronchial cryobiopsy: patchy involvement of fibrosing process next to unaffected lung tissue.

cases (IPF, pulmonary manifestation of scleroderma, see Table 1). This improves the diagnostic yield of TBB for UIP pattern in comparison to data described in literature by up to 50% (see Table 1).

Cryobiopsy specimen tend to be even larger than transbronchial forceps biopsy specimen, and contain more and larger amounts of alveolar tissue [21]. In a previous study the number of alveolar spaces necessary for an adequate biopsy was defined as 20 [6]. This criterion is likely to be fulfilled in most of the cryobiopsy specimen ([21]; unpublished data). However, transbronchial cryobiopsy, as well as TBB by forceps, fail to deliver the diagnosis of UIP in a significant proportion of patients. This may be due to the distance seen frequently between the bronchial wall and typical histological changes, such as fibroblast foci, which are located deeply in the alveolar parenchyma [1] (Figures 1, 2, 3, 4).

Despite the encouraging findings about cryobiopsy these results do not yet command a recommendation of transbronchial cryobiopsy as a standard procedure in the

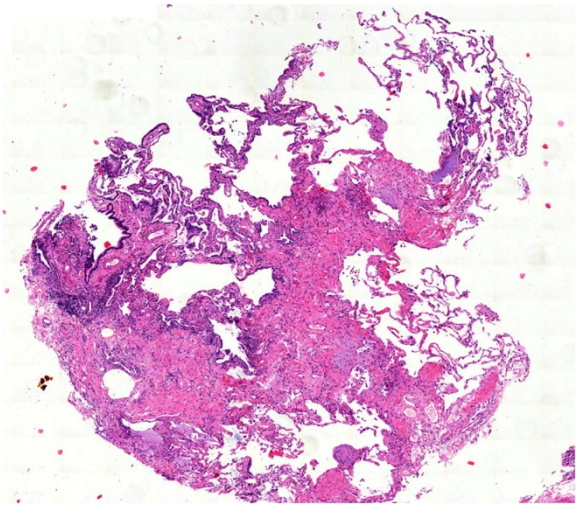

Figure 3 Transbronchial cryobiopsy: architectural distortion of lung tissue with scaring next to normal lung parenchyma.

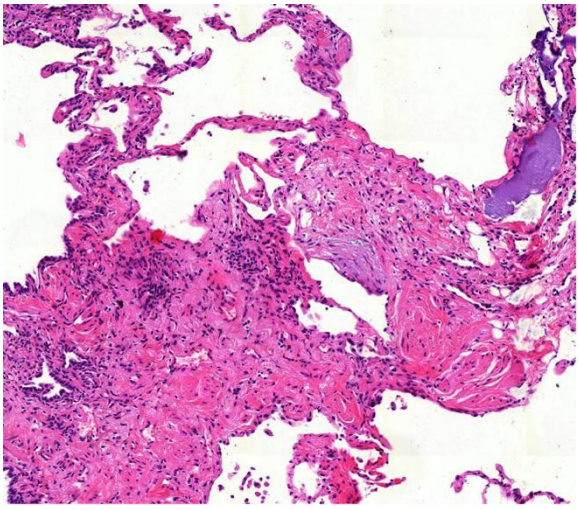

Figure 4 Transbronchial cryobiopsy: active ongoing fibrosis (fibroblast focus) as an expression of "temporary variegation".

processing of suspected pulmonary fibrosis. Nevertheless, the current problem of distinguishing between HP with UIP pattern and IPF (which radiologically speaking cannot be securely discriminated [24]) could be solved by the use of cryobiopsy with greater specimen size. Furthermore, there is a greater chance for detection of granulomas or other characteristic histopathological features [25-29]. Therefore larger prospective and comparative series must be evaluated before a general clinical algorithm can be proposed. Meanwhile, in those individual patients where cryobiopsy has revealed the full pattern of UIP, open lung biopsy is unnecessary if histology and clinical data engender a clear diagnosis.

Conclusions

Cryobiopsy could improve the results reported on conventional transbronchial forceps biopsy. Nevertheless, previously reported series are small and prospective comparisons do not exist. Such studies could even reveal that eventually less cryobiopsy pieces per patient are necessary as compared to transbronchial forceps biopsies. For the latter most of the authors recommend four biopsies per bronchoscopy during the processing of diffuse lung disease. In our present series only 1–2 cryobiopsy specimen were sampled as a rule.

The high diagnostic yield and the lack of any major complication in our series encourages one to proceed with larger studies and to establish transbronchial cryobiopsy within routine clinical algorithms in the diagnostic of diffuse, parenchymal lung disease.

Abbreviations

ATS: American Thoracic Society; COP: Cryptogenic organizing pneumonia; CT: Computed tomography; DPLD: Diffuse, parenchymal lung disease; ERS: European Respiratory Society; HP: Hypersensitivity pneumonia; HR-CT: High resolution computed tomography; IPF: Idiopathic pulmonary fibrosis; NSIP: Non specific interstitial pneumonia; pANCA: Perinuclear anti-neutrophil cytoplasmic antibodies; RB-ILD: Respiratory bronchiolitis associated interstitial lung disease; TBB: Transbronchial biopsy; UIP: Usual interstitial pneumonia.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

SG performed the histological analysis of the samples and drafted the manuscript. NS participated in the design of the study and helped drafting the manuscript. WA performed bronchoscopy, took samples and helped in the coordination of the manuscript. TB performed the statistical analysis. CG performed bronchoscopy and took samples. TB participated in the design of the study and helped in the draft. WG performed bronchoscopy and took samples. TM performed the histological analysis of the samples and helped to draft the manuscript. HW performed bronchoscopy, took samples and drafted the manuscript. All authors read and approved the final manuscript.

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RESEARCH ARTICLE Open Access

Intra-patient comparison of parietal pleural biopsies by rigid forceps, flexible forceps and cryoprobe obtained during medical thoracoscopy: a prospective series of 80 cases with pleural effusion

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Abstract

Background: There is only few data available on the use of cryotechnique during medical thoracoscopy.

Methods: Medical thoracoscopy was performed in consecutive patients with pleural effusion. Prospectively, biopsies were taken by rigid forceps, flexible forceps and cryoprobe. Specimen size, depth and diagnostic yield were compared.

Results: 80 Patients were included. 408 biopsies were taken (205 rigid biopsies, 104 flexible biopsies, 99 cryobiopsies). Mean surface area of rigid biopsies was 22.6 ± 20.4 mm^2 (flexible biopsies: 7.1 ± 9.3 mm^2, cryobiopsies: 14.4 ± 12.8 mm^2). Rigid biopsies were significantly larger than cryobiopsies ($p < 0.001$) and flexible biopsies ($p < 0.001$), crybiopsies were significantly larger than flexible biopsies ($p < 0.01$). A deep biopsy containing fatty tissue was harvested in 63 % of rigid biopsies (cryobiopsy: 49.5 % flexible biopsy: 39.5 %). In 79/80 cases (98.7 % 95 % CI cannot be calculated) a diagnosis was obtained by rigid biopsy (cryobiopsy: 73/80 cases (91.3 % 95 % CI 86.0 – 96.5 %), flexible biopsy: 74/80 cases (92.5 % 95 % CI 88.6 – 97.4 %)). Diagnostic yield achieved with cryobiopsies was inferior to the yield of rigid biopsies (Difference: 12.7 %), but non-inferior to flexible biopsies (Difference: 6.5 %).

Conclusion: Cryobiopsies in medical thoracoscopy are safe with high diagnostic yield, non-inferior to flexible biopsies with increased tissue quantity and quality. Cryotechnique can develop an important role in medical thoracoscopy in the near future when rigid thoracoscopy is not available.

Keywords: Medical thoracoscopy, Cryobiopsy, Parietal pleura, Rigid forceps biopsy, Flexible forceps biopsy

Background

Medical thoracoscopy in rigid and in semi-rigid technique is an efficient and safe procedure in patients with exudative pleural effusion of unknown origin. Biopsy specimen taken during semi-rigid-thoracoscopy are smaller than biopsies taken by rigid forceps but the diagnostic accuracy is said to be similar [1–11]. An advantage of semi-rigid thoracoscopy is flexibility of the endoscope; yet the flexible forceps is smaller and less stable compared to the rigid forceps. Therefore, it is still under debate whether flexible pleural forceps biopsies have the same diagnostic potential as biopsies harvested with rigid forceps.

Cryotechnique was introduced as early as 1968, at first for the therapeutic management of airway diseases [12]. Since then and especially in the last ten years the use of cryotechnique has been established as a routine procedure in bronchoscopy for diagnostic and interventional therapeutic use [13–20]. No increase of complications has been described [21–24]. Furthermore, in diagnostic series it could be demonstrated that central and pulmonary tissue

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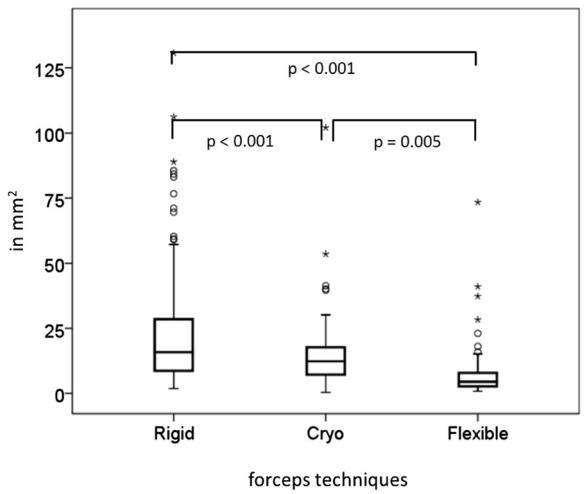

Fig. 1 Total area of specimens taken by rigid forceps, flexible forceps and cryobiopsy (in mm^2, p-values represent results of ANOVA with Bonferroni correction). Boxplots are shown, outliers (values that are between 1.5 and 3 times the interquartile range) are represented by circles beyond the whiskers. Extreme values (values that are more than 3 times the interquartile range) are represented by asterisk beyond the whiskers

samples were larger and better preserved compared to forceps biopsies [25, 26].

The use of cryotechnique in thoracoscopy has been initially described in 1989 [27], and an analgetic advantage was noted 30 years later [28]. Recently, an article describing the feasibility of cryotechnique in medical, semi-rigid thoracoscopy in fifteen patients with exsudative pleural effusion was published [29]. In this series, cryotechnique was shown to be efficient and safe without major complications.

The aim of this prospective study was to compare thoracoscopically obtained parietal pleural biopsies by rigid forceps and flexible forceps to cryobiopsies and to document the non-inferiority of cryotechnique in this procedure.

Methods

Patients

This prospective study was performed at a tertiary respiratory care center (Lungenklinik Heckeshorn) between 2012 and 2014. All consecutive patients with exsudative pleural effusion of unknown etiology with indication for medical thoracoscopy were eligible for this study. Prior to the thoracoscopy and study enrollment, all patients were informed and written consent was obtained. The study was approved by the medical ethics committee of the Charité – Universitätsmedizin Berlin (Ethikkommission, Ethikausschuss 4 am Campus Charité – Mitte).

Thoracoscopy and forceps techniques

Medical thoracoscopy with rigid single-port-of-entry technique was performed in the endoscopy suite under local anesthesia and sedation as described elsewhere [30]. All procedures were performed using a rigid thoracoscope (11 mm, Storz, Tuttlingen, Germany). To define the point of entry into the pleural cavity, an ultrasound was carried out followed by the introduction of a pneumothorax under fluoroscopic guidance. Under direct vision with the thoracoscope, all pleural fluid was removed and the pleural cavity was inspected. Afterwards, specimen were taken with rigid forceps (3 mm, Storz, Tuttlingen, Germany), flexible forceps (2.8 mm, Boston Scientific Radial Jaw, Natick, MA, USA) and cryoprobe (2.4 mm, Erbokryo CA, Erbe, Tübingen, Germany) in random order. Cryotechnique takes advantage of the Joule-Thompson-Effect (rapid gas release with high flow induces low temperature up to -77° Celsius); this leads to freezing and adhesion of specimen and parietal pleura. At last, the attached tissue was extracted together with the cryoprobe. In cases of obvious malignancy a pleurodesis with talcum poudrage was performed. After the procedure, a chest tube was placed into the pleural cavity.

Morphometrical and morphologic analysis of thoracoscopic biopsies

All biopsies were processed conventionally by serial sectioning of at least 12 Hematoxylin-eosin (HE) stained section steps as it refers to be the standard procedure analyzing pleural biopsies in histological routine as described elsewhere [25, 26]. Serial sectioning is therefore used to avoid incomplete sectioning of particles in order to provide a valid histopathological diagnosis. All biopsies were subsequently surveyed regarding size and quality. The pathologist was not blinded regarding to the technique used to obtain the biopsy.

The HE stained slides were therefore scanned by a ZEISS-MIRAX Midi Slide scanning system using the Mirax Viewer Image Software Version 1.12 (Zeiss Microimaging, Oberkochen, Germany and 3D Tech, Budapest, Hungary). Regarding biopsy size the total area was measured by interactive circling of the largest biopsy section of each serial. All areas were calculated automatically and provided in mm^2. Biopsy quality was determined by tissue depth. A thoracoscopic biopsy specimen including fatty tissue of the thoracic wall was considered to be a deep biopsy and therefore of high quality.

Statistical analyses

For data analyses a statistical software (Statistical Package for Social Sciences, Version 22.0; SPSS, Chicago, IL, USA) was used on a Windows XP operating system (Microsoft; Redmond, WA, USA). Results were expressed as

Table 1 Comparison of forceps techniques concerning number of biopsies and inclusion of fatty tissue

	Number of biopsies	Positive for fat tissue	Negative for fatty tissue	Biopsies incl. fatty tissue (%)
Rigid forceps biopsy	205	129	76	62.9
Flexible forceps biopsy	104	41	63	39.4
Cryoprobe biopsy	99	49	50	49.5

frequencies or as mean ± SD. The multiple comparison of surface area of the three methods (rigid, flexible, and cryobiopsy) were performed by ANOVA with post-hoc Bonferroni correction.

The following method was used to weigh observed differences between diagnostic success rates to establish a histopathological diagnosis. Two-sided 95 % confidence intervals (CI) for single proportions were calculated according to a standard formula ($CI = p \pm Z_{\alpha/2} \times \sqrt{[(p \times q)/n]}$) for all proportions (cases diagnosed (x) over all cases (n)) when $x - n \geq 5$. We used this statistical method to assume non-inferiority for the differences in success rates. Non-inferiority was assumed if the difference of the lower limit of this confidence interval of the inferior method was not larger than 10 % compared to the highest observed success rate (assumed this to be higher than 90 %).

The significance level of the analyses was set to 5 %, and exact *p* values were reported were appropriate.

Results

Eighty patients with a mean age of 67.5 ± 13.5 years were included in the study. For each patient, three to four biopsies by rigid forceps were taken. Due to the duration of the procedure, one to two cryobiopsies were taken as well as one to two biopsies by flexible forceps. Altogether, 408 biopsies were taken and analyzed (205 biopsies by rigid forceps, 104 biopsies by flexible forceps, and 99 biopsies by cryoprobe).

Table 2 Overview of histopathological diagnosis and the number of cases

Histopathological diagnosis	Number of cases (%)
Idiopathical chronic pleuritis	33/80 (41%)
Non-small cell lung cancer	19/80 (24%)
Pleural carcinomatosis by breast cancer	11/80 (14%)
Lymphoma	4/80 (5%)
Pleural carcinomatosis by other solid malignoma	4/80 (5%)
Malignant mesothelioma	3/80 (4%)
Tuberculous pleurisy	3/80 (4%)
Small cell lung cancer	2/80 (3%)
Asbestosis	1/80 (1%)
Total	80/80 (100%)

The mean surface area of biopsies taken with the rigid forceps was 22.6 ± 20.4 mm^2, with the flexible forceps 7.1 ± 9.3 mm^2, and with the cryoprobe 14.4 ± 12.8 mm^2 (Fig. 1). Rigid forceps biopsies were significantly larger than samples taken by cryoprobe ($p < 0.001$) and flexible forceps ($p < 0.001$). Biopsies by cryoprobe were significantly larger than flexible forceps biopsies, too ($p < 0.01$; one-way ANOVA, with Bonferroni correction).

A deep biopsy containing fatty tissue was obtained in 63 % of the rigid forceps biopsies, in 49.5 % of the samples harvested with the cryoprobe and in 39.5 % of the biopsies by flexible forceps (see Table 1).

In 66 out of all 80 cases (83 %) a deep biopsy was obtained by using the combination of all three described methods.

Histopathologic diagnoses

In the histopathological work-up, 43/80 malignant (54 %) and 37/80 non-malignant (46 %) diagnoses were found (see Table 2).

In 79/80 cases (98.7 %, 95 %CI cannot be calculated) a diagnosis was obtained by rigid forceps biopsy. This was true in 73/80 cases (91.3 %, 95 % CI 86.0 – 96.5 %) for cryoprobe samples and in 74/80 cases (92.5 %, 95 %CI 88.6 – 97.4 %) for biopsies taken by flexible forceps. According to the assumptions made for non-inferiority, the diagnostic yield achieved with cryobiopsies was inferior to the yield rigid forceps biopsies (Difference: 12.7 %), but non-inferior to the flexible method (Difference: 6.5 %). The diagnostic yield was also different between samples harvested with rigid or flexible forceps (Difference: 10.1 %), therefore non-inferiority could neither be established for this comparison.

Analyzed per patient, in 73/80 cases (91 %) all three forceps techniques showed the concordant histopathological result. In 3/80 cases (4 %), only the samples gained by rigid forceps showed the diagnostic histology. In 1/80 case (1 %) an idiopathic pleuritis turned out to be a malignant mesothelioma after 12 month follow-up which was not detected in the thoracoscopic samples during the study.

Complications of the procedure

No complications such as bleeding or pain occurred during the procedure in any of the biopsy techniques. Furthermore, after the procedure no complications such as empyema or prolonged fistula were noted.

Discussion

This prospective study compared the two established biopsy techniques (rigid and flexible forceps biopsy) with the use of cryotechnique during medical thoracoscopy.

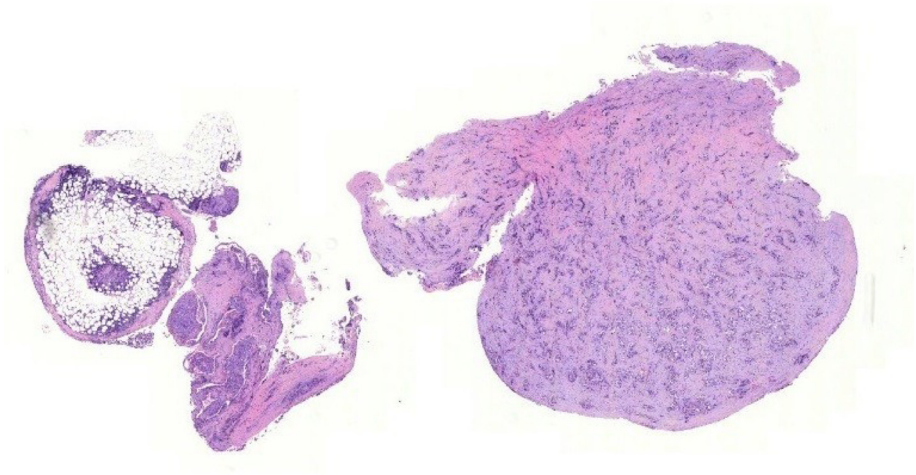

Fig. 2 Histological comparison of biopsy techniques: diagnosis of pleural manifestation of a pulmonary, TTF1-positive adenocarcinoma

In comparison, cryobiopsies showed a significantly larger biopsy size and depth than flexible forceps biopsies. On the other hand, rigid forceps biopsies showed, as expected, the significantly largest size and depth and the highest diagnostical yield. As cryotechnique can be used during semi-rigid thoracoscopy, and if a rigid forceps biopsy is not available, one could speculate that this inexpensive technique could overcome the problem of a too small size of flexible forceps. Furthermore, important for the pathologist was the fact that samples gained by cryotechnique showed less tissue damage in an overall very good quality (Figs 2, 3, 4 and 5) which was already described for lung biopsies [25, 26].

However, in the histopathological work-up, all three biopsy methods had a diagnostic yield of more than 90 %; in 91 % of the cases the three different techniques showed the same histologic result. In one patient, after a 12-month-follow-up that was carried out for all patients, a malignant mesothelioma was detected, that had not been found in any of the three techniques during medical thoracoscopy. This data demonstrates the non-inferiority concerning diagnostic (histological) accuracy of cryotechnique only in comparison to flexible forceps biopsy.

Furthermore, our study demonstrated the safety of the use of cryotechnique during medical thoracoscopy. No biopsy-related complications such as major bleeding or pain after tearing the probe were noted. These findings confirm the preliminary data by Rozman et al. [29], describing parietal pleural biopsies obtained by cryoprobe as safe.

In this series of 80 patients the histological diagnosis of malignant mesothelioma was only detected three times. Especially for this diagnosis the depth and quality of the biopsy is extremely important [31]. As a next step, a larger series including more patients suffering from malignant mesothelioma should be examined in a multicenter study, as this diagnosis is the

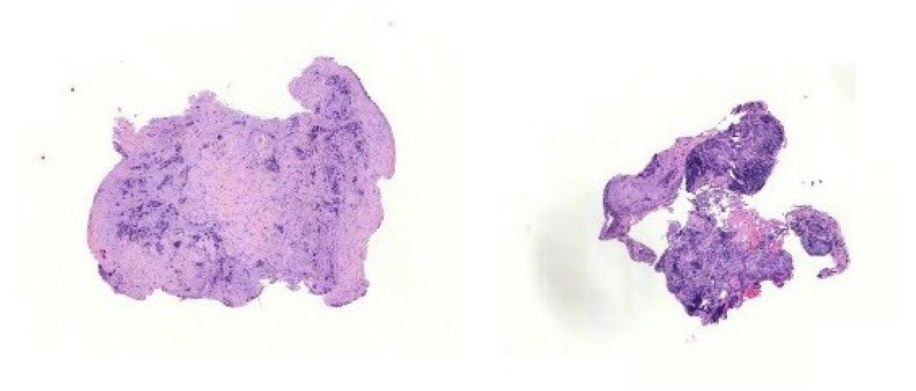

Fig. 3 Flexible forceps biopsy of a pleural carcinomatosis of pulmonal adenocarcinoma: pleural connective tissue and desmoplastic stroma (*left side*) with infiltrates of a non-small cell carcinoma (size 3,88mm^2)

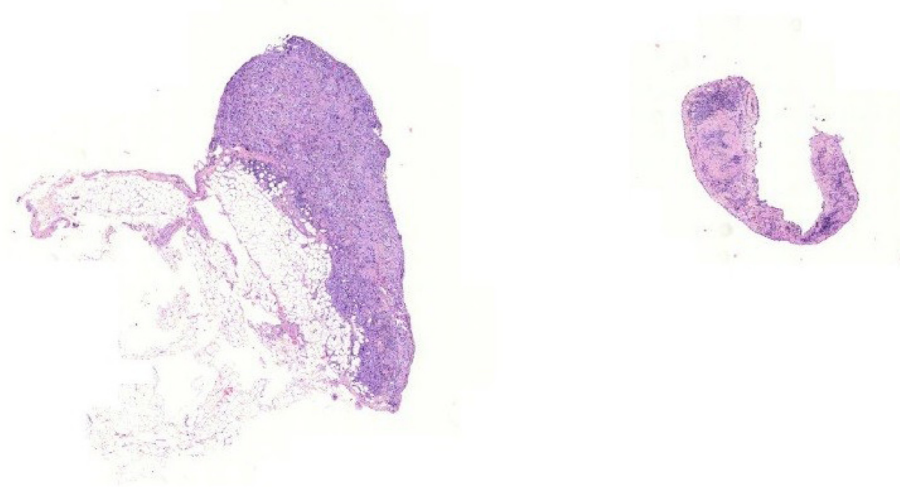

Fig. 4 Cryobiopsy of a pleural carcinomatosis of pulmonal adenocarcinoma: pleural fat tissue (left side) and pleural connective tissue (*right side*) with infiltrates of a non-small cell carcinoma (size 11,57mm^2)

most frequently missed by medical as well as surgical thoracoscopy [32]. As a hypothesis, the higher number of deep biopsies containing fatty tissue should enable to detect mesothelioma with a higher yield of cryobiopsy compared to flexible forceps biopsy [31].

Conclusion

In summary, cryobiopsy obtained during medical thoracoscopy is a safe method with high diagnostic value, comparable to flexible forceps biopsy, but inferior to rigid forceps biopsies. Samples are smaller and less deep than rigid forceps biopsies, but significantly larger and deeper than samples gained by flexible forceps. Therefore, the use of cryobiopsy in semi-rigid thoracoscopy can not yet be generally recommended to replace rigid forceps biopsies during medical thoracoscopy.

The quantity of harvested tissue becomes increasingly important, because personalized therapy concepts especially for non-small cell lung cancer and breast cancer

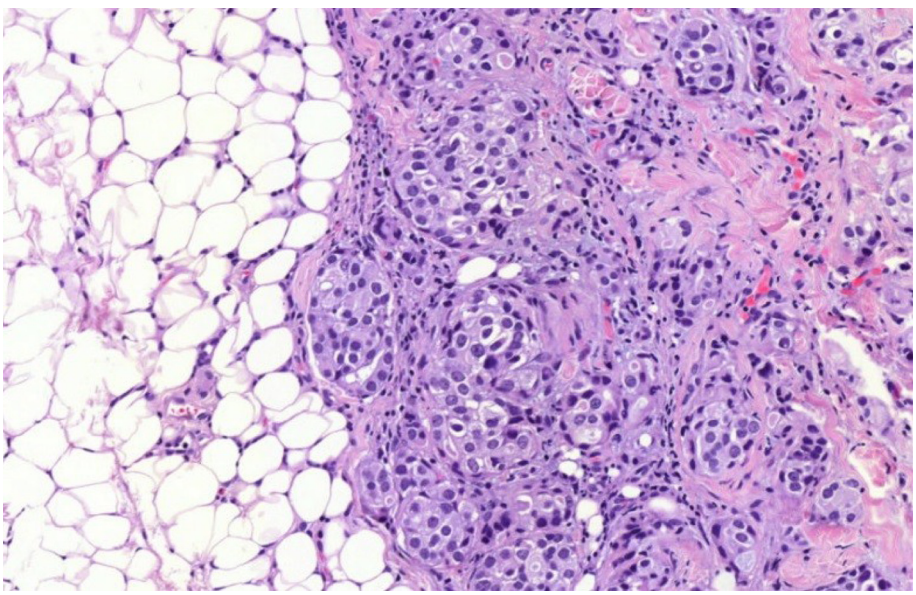

Fig. 5 Magnification of the cryobiopsy in Fig. 3: fat tissue *(left side)*, which is infiltrated by tumor nests with glandular differentiation (*right side*), a manifestation of an adenocarcinoma respect

demand more pathologic investigations. This will favour the use of cryotechnique where rigid forceps biopsies are not available or cannot be used.

Abbreviations
CI, confidence interval; HE, hematoxylin-eosin; mm, millimeter; SD, standard difference

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The datasets supporting the conclusions of this article are completely included within this article.

Authors' contributions
HW performed thoracoscopies and wrote the main part of the paper. NS corrected the paper. MB performed thoracoscopies. TB corrected the paper and worked out the statistical analysis. CD performed thoracoscopies. RS corrected the paper and took part in the histopathological and statistical analysis. TM performed histopathological analysis and corrected the paper. SG performed histopathological analysis and took part in writing and correcting the paper. All authors read and approved the final manuscript.

Competing interests
Henrik Wurps, Nicolas Schönfeld, Torsten T. Bauer, Mathias Bock, Christian Duve, Rica Sauer, Thomas Mairinger and Sergej Grif declare that they have no financial or non-financial competing interests.

Consent for publication
Does not apply to this particular manuscript.

Ethics approval and consent to participate
Prior to thoracoscopy and study enrollment, all patients were informed and written consent for participation and publication of the data was obtained. The study was approved by the medical ethics committee of the Charité – Universitätsmedizin Berlin (Ethikkommission, Ethikausschuss 4 am Campus Charité – Mitte).

Endnotes
Does not apply to this particular manuscript.

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Arbeit 5

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Journal of Medical Case Reports

CASE REPORT Open Access

Diffuse idiopathic pulmonary neuroendocrine cell hyperplasia diagnosed by transbronchial lung cryobiopsy: a case report

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Abstract

Background: Micronodular lesions are common findings in lung imaging. As an important differential diagnosis, we describe a case of diffuse idiopathic pulmonary neuroendocrine cell hyperplasia; it is notable that the diagnosis of diffuse idiopathic pulmonary neuroendocrine cell hyperplasia is often delayed. This case provides supporting evidence to establish lung biopsy by cryotechnique as the option of first choice when considering a diagnostic strategy for micronodular lung lesions.

Case presentation: We report a case of a 65-year-old white woman who presented with obstructive symptoms of chronic coughing and dyspnea confirmed by conventional lung function tests. A computed tomography scan presented disseminated micronodules in all the lobes of her lungs. With the help of bronchoscopic cryobiopsy it was possible to obtain a high yield sample of lung parenchyma. On histologic examination, the micronodules correlated with a diffuse neuroendocrine cell hyperplasia. In the context of clinical symptoms, radiological aspects, and histomorphological aspects we made the diagnosis of a diffuse idiopathic pulmonary neuroendocrine cell hyperplasia. Obstructive symptoms were treated with inhaled steroids and beta-2-mimetics continuously. A comparison between current computed tomography scans of our patient and scans of 2014 revealed no significant changes. Last ambulatory checks occurred in January and May of 2016. The course of disease and the extent of limitation of lung function have remained stable.

Conclusions: The diagnosis of diffuse idiopathic pulmonary neuroendocrine cell hyperplasia is best made in a multidisciplinary review including clinical presentation, lung imaging, and histomorphological aspects. This report and current literature indicate that transbronchial lung cryobiopsy can be used as a safe and practicable tool to obtain high quality biopsies of lung parenchyma in order to diagnose micronodular lesions of the lung.

Keywords: Case report, DIPNECH, Obliterative bronchiolitis, Cryobiopsy

Background

Diffuse idiopathic pulmonary neuroendocrine cell hyperplasia (DIPNECH) is a rare idiopathic disease, which was named by Aguayo *et al.*; it is associated with neuroendocrine cell hyperplasia and obliterative bronchiolitis [1]. Patients are typically older women and non-tobacco smokers who are affected by obstructive symptoms such as coughing and dyspnea [2]. On histologic examination, DIPNECH presents as a scattered nodular or linear proliferation of neuroendocrine cells either superficial to the basement membrane of bronchial or bronchiolar epithelium or in the form of tumorlets beyond it. Tumorlets are neuroendocrine cell proliferates smaller than 5 mm in diameter. The World Health Organization defines DIPNECH as a praeneoplastic condition. Patients may develop synchronous or subsequent carcinoid tumors, which measure more than 5 mm whereas tumorlets measure

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less than 5 mm [3, 4]. DIPNECH is known to have a good prognosis. Most cases show stability in symptoms and radiological findings. Therefore a watch and wait strategy seems the method of choice. However, a few patients show rapid clinical progression [2]. In cases of progressive disease a surgical excision of dominant lesions and somatostatin analogs may be considered a therapy option [5]. Similar to other obstructive lung diseases, complications such as acute exacerbation including bronchitis and pneumonia can occur. A standard procedure in diagnostic strategy and therapy of DIPNECH has not yet been established.

Case presentation

In November 2014, a 65-year-old white woman with a history of progressive dyspnea presented to our hospital for evaluation. She complained about dyspnea on exertion and had had several infectious exacerbations of chronic obstructive pulmonary disease (COPD) during the last year, one requiring hospitalization. During those exacerbations she had been treated with tapering doses of systemic corticosteroids showing improvement in hypoxemia and obstructive symptoms. At the point of presentation her medication for inhalation included: budesonide/formoterol 400 μg/12 μg twice a day, tiotropium bromide 18 μg once a day, and salbutamol as needed, which she was using at least once a day. Her general patient history was negative for atopic diseases or allergies. At the age of 54 she was diagnosed as having COPD due to dyspnea and typical lung function tests. She stated that she had never smoked tobacco. A skin prick test revealed no hypersensitivity. Since the diagnosis of COPD had been established she was regularly followed-up by a pulmonologist. At hospital admission, her general condition was slightly disturbed. She was hypoxic at rest. The saturation level of oxygen in hemoglobin (SaO_2) was 92%, without providing indication for oxygen therapy.

A body plethysmography showed forced vital capacity (FVC) 1.47 L (62%), forced expiratory volume in 1 second (FEV_1) of 0.8 L (40%), airway resistance of 1.62 kPa × second/L (538.9%), residual volume of 3.30 L (188%), and a total lung capacity of 4.50 L (108%). Those results were evaluated as severe obstruction with massive air trapping compatible with the diagnosis of COPD. During the further evaluation process different methods of imaging were conducted. A chest X-ray showed well-ventilated lungs and discreet apical pleural callosity. Transthoracic echocardiography showed normal cardiac structures without evidence of pulmonary hypertension. In a computed tomography (CT) scan, disseminated small nodules between 2 and 4 mm in all lobes, ground glass characteristic, and partial mosaicism on both lungs were strikingly apparent. Focal bronchial thickening could be seen. Furthermore, mediastinal lymph nodes were heightened (Fig. 1).

As a result of lung imaging, bronchoscopy was initiated. Forceps biopsies of her central airways were taken, showing normal respiratory mucosa without any changes suspicious for a specific inflammation. In particular, granulomas or giant cells could not be detected. Due to ongoing clinical suspicion for interstitial lung disease a further attempt of forcing a histological diagnosis was made.

By method of transbronchial cryobiopsy (ERBE-CRYO2, diameter 1.9 mm; Tübingen, Germany), lung tissue samples of the middle and inferior lobes of her right lung were gained. The material was formalin fixed and paraffin embedded, cut into 4 μm-thick sections and stained with hematoxylin and eosin (H&E). The fragmented biopsy measured an overall area of 30.9 mm^2 compared with the initial biopsy of 7.5 mm^2 (Mirax Viewer Image Software Version 1.12, Zeiss Microimaging, Oberkochen, Germany and 3D Tech, Budapest, Hungary). Besides small airways, a regular lung parenchyma with an alveolar basic structure could be seen. The bronchioles were lined by a regular respiratory epithelium. Furthermore, in her bronchiolic mucosa, linear and nodular proliferates of small uniform cells with round to slightly ovoid nuclei and disperse chromatin were located within the epithelial basement membrane and bulged into the lumina (Fig. 2). Peribronchiolar and perivascular aggregates of cells showing this morphology were also found, measuring less than 5 mm in diameter at maximum. No signs of malignancy could be found; neither could we find any mitotic activity, desmoplastic stroma reaction, or any invasive aspect. In association with the described cell cluster, slight fibrosis could be

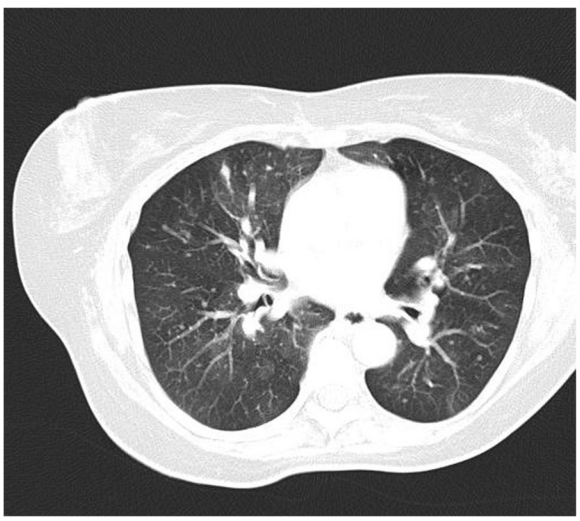

Fig. 1 Axial image from chest computed tomography scan showing multiple scattered pulmonary nodules and mosaicism

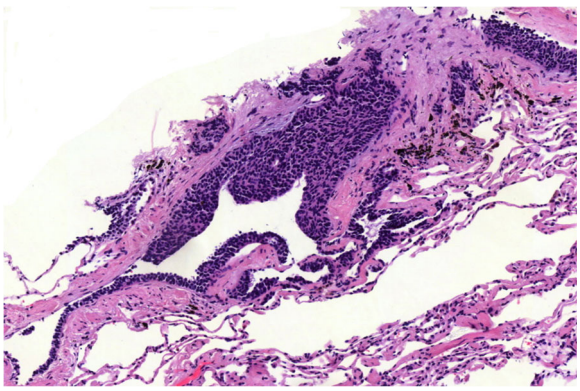

Fig. 2 Hematoxylin and eosin stained slide shows bronchiole with linear and nodular proliferates of neuroendocrine cells bulging into the lumen

seen. In the Elastica van Gieson stain, the walls of some bronchioles were broadened and contained an increased amount of elastic fibers. Immunostainings revealed strong positivity for synaptophysin (Fig. 3), chromogranin A, and CK7 in the cell cluster described. The proliferative index determined by Ki67% (Mib-1) was beneath 1% (antibodies by Roche, Rotkreuz, Switzerland). Based on these findings, the diagnosis of a neuroendocrine cell hyperplasia could be made and a possible association with DIPNECH was noted.

Due to concomitant bronchitis our patient received antibiotic therapy. Obstructive symptoms were treated with inhaled steroids and beta-2-mimetics continuously. Comparing current CT scans of our patient with scans in 2014 no significant changes are described. Last ambulatory checks occurred in January and May of 2016. The course of disease and the extent of limitation of lung function have remained stable.

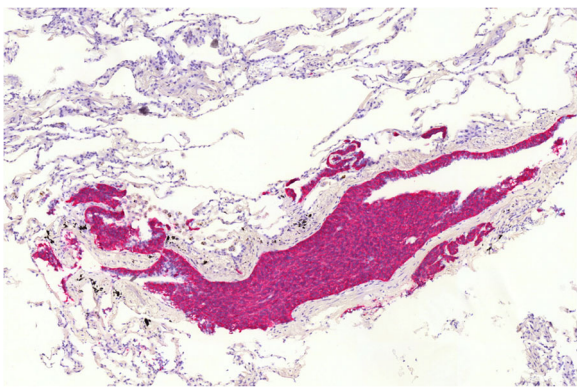

Fig. 3 Strong positivity in immunohistochemical analysis with synaptophysin

Discussion

On the background of clinical presentation and lung imaging the microscopic morphology is compatible with a manifestation of DIPNECH. Our patient presented has gone through a history of obstructive lung disease including symptoms like exertional dyspnea with slight relief under anti-obstructive therapy. Also, lung function tests have repeatedly shown severe obstruction. In comparison to a typical patient who has COPD, our patient does not have a history of tobacco smoking. In the past and recent literature, DIPNECH has been described to be potentially associated with obliterative bronchiolitis and respectively could be the cause of chronic obstruction [1, 2, 6, 7].

On histologic examination, the wall of her bronchioles were thickened and showed a linear and nodular neuroendocrine cell hyperplasia, in which an incomplete obliteration of these airways could be shown by cell bulging into the airway lumen. However, the complete morphological picture of an obstructive bronchiolitis is not comprised in the obtained biopsy. However, it can easily be imagined that even an incomplete obliteration of bronchioles can cause obstruction comparable to symptoms induced by a follicular bronchiolitis, in which prominent lymphatic tissue obliterates bronchiolic lumina. Furthermore, broncho-obstruction can be caused or at least aggravated by different vasomodulating and bronchomodulating peptides produced in hyperplastic/neoplastic pulmonary neuroendocrine cells [8]. Any of these changes may have caused the obstructive symptoms of this patient, including typical lung function tests, potentially leading to earlier diagnoses of chronic obstructive lung disease or intrinsic asthma. In this regard, it is notable that the final diagnosis of DIPNECH is often delayed [7].

Micronodular lesions of the lung can be coincidental diagnostic findings in lung imaging. Also, DIPNECH is sometimes asymptomatic and may be diagnosed accidentally [2]. To consider different courses of disease the term “DIPNECH syndrome” has been proposed [9]. In CT imaging DIPNECH can present not only with multiple small nodules in both lungs but also with bronchial wall thickening and mosaic perfusion [10]. Rare radiological findings include fibrotic changes comparable to a restrictive pattern in usual interstitial pneumonia [11]. In addition to lung imaging, surgical lung biopsies are usually required to diagnose DIPNECH and corresponding obliterative bronchiolitis [6]. Any other symptomatic or asymptomatic micronodular lesion, for example sarcoidosis, usually requires an open lung biopsy to be clarified as well. Further diagnostic tools like an octreotide scan and positron emission tomography (PET)-CT are also helpful. Blood examinations might reveal elevated serum levels of chromogranin A [5].

In this special case, transbronchial cryobiopsy was used to obtain a good quality biopsy of lung parenchyma from which it was possible to generate the diagnosis of DIPNECH. This method has an advantage over the use of conventional transbronchial forceps because alveolar tissue is contained more often and shows fewer crush artefacts [12, 13]. Besides, the cryotechnique method seems safer and less straining for patients compared to open biopsy methods [14]. The risk of significant bleeding in transbronchial cryobiopsy does not differ from that of using conventional forceps [15]. In particular, concerning histomorphological analysis of interstitial lung diseases, the cryotechnique can be used in combination with flexible catheters to reach peripheral areas of the lung [16]. Transbronchial cryobiopsy has a particularly good diagnostic yield for diseases diffusely involving the lung parenchyma [17, 18]. In summary, transbronchial cryobiopsy can be regarded as an ideal method for diagnosing DIPNECH.

Conclusions

The diagnosis of DIPNECH is best made in a multidisciplinary review including clinical presentation, lung imaging, and histomorphological aspects. Current literature indicates that transbronchial lung cryobiopsy can be used as a safe and practicable tool to obtain high quality biopsies of lung parenchyma in order to diagnose micronodular lesions of the lung. Therefore, this case provides supporting evidence to establish lung biopsy by cryotechnique as the option of first choice before using surgical biopsy techniques.

Abbreviations

COPD: Chronic obstructive pulmonary disease; CT: Computed tomography; DIPNECH: Diffuse idiopathic pulmonary neuroendocrine cell hyperplasia; FEV_1: Forced expiratory volume in 1 second; FVC: Forced vital capacity; H&E: Hematoxylin and eosin; PET: Positron emission tomography; SaO_2: Saturation level of oxygen in hemoglobin

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Availability of data and materials

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Authors' contributions

RS was involved in the literature review and wrote the case report. SG was also involved in the literature review. SG and TM performed the histological examinations and measurements on samples. SG, TM, and AB helped drafting the manuscript. AF, AB, and CG were involved in the management of the case, provided relevant history and laboratory results. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

Consent for publication

Written informed consent was obtained from the patient for publication of this case report and any accompanying images. A copy of the written consent is available for review by Editor-in-Chief of this journal.

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LEBENSLAUF

Aus datenschatzrechtlichen Gründen wird mein Lebenslauf in der elektronischen Version meiner Arbeit nicht veröffentlicht.

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