Non-Specific Pleuritis: pathological patterns in benign pleuritis

Georgia Karpathiou[1], Sirine Hathroubi[1], Arnaud Patoir[2], Olivier Tiffet[2], Francois Casteillo[1], Clement Brun[3], Fabien Forest[1], Najib M. Rahman[4], Michel Peoc'h[1], Marios E. Froudarakis[3]

Departments of Pathology[1], Thoracic Surgery[2], Pneumonology and Thoracic Oncology[3] North Hospital, University Hospital of St-Etienne, France and [4]Oxford Respiratory Trials Unit, University of Oxford UK

Corresponding author:

Georgia Karpathiou MD, PhD, BSc
Department of Pathology
University Hospital of Saint-Etienne
42055 CEDEX2 St-Etienne, France
e-mail: gakarpath@yahoo.gr

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Abstract

Background: A pleural biopsy without granulomatous inflammation or tumor cells is interpreted as “non-specific pleuritis” (NSP), a diagnosis without any specificity, often frustrating for physicians. However, varying histological features are found in NSPs with unknown significance. **Objectives**: To describe the detailed microscopic features of NSP and correlate them with the underlying etiology. **Material and methods**: 100 patients diagnosed with NSP after pleural biopsy, were retrospectively evaluated. A benign cause of pleural effusion was attributed. Histological features evaluated were inflammation, fibrosis, vascular proliferation, haemorrhage, fibrin, oedema and mesothelial hyperplasia. A semi-quantitative scoring was applied. **Results**: Bacterial-caused and autoimmune disease-associated NSPs showed the higher score followed by viral and drug-induced conditions, while pneumothorax and cardiac-induced NSP showed the lower score ($p<0.0001$). The degree of fibrosis was higher in bacterial NSP, and the type of fibrosis was cellular in this group ($p=0.006$). Vascular proliferation differed between groups ($p<0.0001$), with bacterial NSP showing the higher one. **Conclusion**: Histological findings differ significantly between the varying etiologies of NSP, and this may be used to suggest ~~identify~~ the cause of the effusion.

Introduction

The pleural cavity is a virtual space surrounding the lungs and containing a very small amount of fluid, normally less than 15 mL in a 70-kg individual (1) (2). An abnormal collection of fluid within this cavity is called pleural effusion. Pleural effusion is a common presenting feature of a wide range of pleural, pulmonary or systemic diseases, benign or malignant. Standard work-up in this case includes clinical history, physical examination, blood tests, diagnostic thoracentesis, chest radiograph and computerized tomography. Thoracoscopy will be often performed as it provides the possibility to obtain pleural tissue under direct inspection of the pleural cavity (3). Diagnostic sensitivity of thoracoscopy for malignancy reaches 95% (3).

However, in cases where a malignancy or a granulomatous disease is not histologically diagnosed, microscopic features for the rest of possible etiologies are considered largely the same, and a descriptive type of histopathological diagnosis will be employed for most of them. The term kept for these cases is “non-specific pleuritis” (NSP). After thoracoscopy, a diagnosis of NSP is rendered in 8% to 38% of the cases, with 25% of them considered 'idiopathic” with no probable cause, while almost 10% of these cases will finally reveal malignancy, particularly mesothelioma (4) (5) (6) (7). This reveals the difficulties in establishing the correct diagnosis, histologically and clinically, in NSP cases but it also highlights an important absence in our knowledge concerning this entity: there are no studies describing in detail the microscopic features of NSP and their potential correlation with the underlying etiology.

Thus, the aim of the study is to report the detailed histological features of a NSP series in order to define differences in the histopathological pattern according to the underlying disease.

Material and Methods

Study group

This study used information extracted from the medical electronic files of 100 consecutive patients diagnosed with NSP after pleural biopsy for recurrent undiagnosed pleural effusion at the University Hospital of Saint-Etienne from January 2014 to January 2017. Inclusion criteria were (a) a histological diagnosis of NSP excluding cases of neoplastic or granulomatous disease and (b) a minimum follow up of 12 months (8). The study was approved by the Internal Review Board of the University Hospital of Saint-Etienne (212018/CHUSTE).

Medical files were reviewed to record demographic data, clinical features, previous clinical history and comorbidities, asbestos exposure, medications and additional laboratory and investigational data. The predominant macroscopic appearence of the pleural cavity, recorded by the thoracoscopist was also documented as: inflammatory, fibrinous, septated, hemorrhagic or pleural plaques. All data were reviewed clinically to achieve a final etiological diagnosis.

Histopathological evaluation

All available slides were reviewed to evaluate the following parameters: (a) inflammation, (b) fibrosis, (c) vascular proliferation (d) edema, (e) fibrin, (f) hemorrhage, (g) mesothelial hyperplasia. As no histological scoring system concerning the pleura has been established to date in humans, we used a system of an experimental model of pleural disease (9) (10). According to it, a semi-quantitative system of 0=absence, 1=mild, 2=moderate, 3=severe is applied for each parameter. A final score was attributed as the sum of all values (10).

Furthermore, concerning inflammation, the presence of all different cellular types was recorded for each case and also the predominant inflammatory cell type

(lymphocytes, plasmacytes, histiocytes, neutrophil granulocytes, eosinophil granulocytes) was separately recorded. Likewise, concerning fibrosis this was further characterized into the following types: (a) cellular, when a fibroblast-rich type fibrosis was found, (b) old, when only a well-established collagenous fibrosis poor in cells was found and (c) mixed, when both types of fibrosis were found. Moreover, the presence of prominent layering of tissue elements was recorded.

Statistical analysis

Data were analyzed using the Stat View software (Abacus Concepts, Berkeley Ca, USA). We used the chi-square test to explore any relationship between two groups for categorical data, factorial analysis of variances (ANOVA) to consider the effect of at least one factor on a continuous parameter studied. For all analyses, statistical significance was set at a p value of < 0.05.

Results

Demographics

Patients' demographics are shown in Table 1. Age at diagnosis ranged from 13 to 94 years with a median of 63 years. Seventy-one patients were male and 29 were female. Median follow-up was 36 months. Eight patients died during the post-operative period or follow-up, none due to malignancy-associated pleural effusion. Overall, no pleural malignancy was revealed during follow-up.

The predominant macroscopic pattern during thoracoscopy was inflammatory in 40% of the cases, followed by fibrinous (14%), septated (8%), fibrous (6%) and hemorrhagic (2%). Pleural plaques, as the predominant pattern, were found in two patients (2%), whereas another 13 patients harbored histologically confirmed plaques.

Regarding final etiological diagnosis based to all available data, this included pneumothorax in 28 patients (28%) and bacterial infection in 27 patients (27%). In the

latter case, the following bacterial species were found: *Pnemococcus, Staphylococcus, Streptococcus, Pseudomonas, Hemophilus, Escherichia, Neisseria*. Viral pleural effusion was separately categorized and included 7 patients (7%). Cardiac-associated pleural effusion were 10 (10%), drug-induced 7 (7%) and of auto-immune etiology 5 (5%). Cases merged into the "Other" group (n=16, 16%), in terms of analysis, included: cases attributed to asbestos exposure (n=2), chylothorax (n=1), post-traumatic (n=2), pulmonary embolism (n=1), Dressler syndrome (n=1), post-irradiation (n=1), kidney failure (n=1), eosinophilic pleuritis (n=1), and in six cases (6%) no possible etiology was attributed. These 16 cases were excluded from further statistical analysis due to the very limited number with specific etiology or their unknown etiology.

Clinicopathological correlations

Histological findings are summarized in Table 2 and illustrated in Figures 1-3. Association of histological findings with effusion's aetiology is presented in Table 3. The higher final score of pathological changes was found in bacterial diseases and autoimmune ones; viral and drug-induced NSPs showed a similar moderate score while the lower score was produced by heart disease and pneumothorax ($p<0.0001$).

Seen by histological feature, degree of inflammation did not differ between the different etiological groups ($p=0.3$) but the predominant inflammatory cell type did differ, as in most groups lymphocytes was the predominant type while in the pneumothorax and the bacterial group, histiocytes and neutrophils, respectively, were also found ($p= 0.02$). The presence of histiocytes, was a feature mostly of pneumothorax and viral disease ($p<0.0001$), in comparison to other etiologies.

Degree of fibrosis differed between etiological groups as the bacterial-caused group showed the most severe fibrosis ($p=0.03$). Furthermore, the type of fibrosis differed as it was cellular in the bacterial-caused group in comparison to other groups, which showed an

old type of fibrosis (p=0.006). Similarly, layering of tissue elements was more often seen with bacterial pleural effusion than the rest of the groups (p=0.05). Vascular proliferation and hemorrhage were mostly features of bacterial infection (p<0.0001 and p=0.02, respectively). Edema presence did not differ between categories (p=0.1). Mesothelial hyperplasia was more often found with pneumothorax (p=0.009).

The total final score was associated with the predominant macroscopic pattern (p=0.002), as septated and hemorrhagic patterns showed the higher final score (mean 5.2±0.8 and 6.5±0.7, respectively), followed by fibrotic, fibrinous and inflammatory (mean 4.5±1.7, 4.7±1.3 and 4.4±1.5, respectively), while pleural plaques aspect showed the lower one (2.5±2.1).

Vascular proliferation was significantly associated with fibrosis (p<0.0001), as it was present in biopsies with severe and moderate fibrosis and not with mild or no fibrosis. It was also significantly associated with the type of fibrosis (p<0.0001) as it was the cellular type that was mostly characterized by vascular proliferation. Vascular proliferation was not associated with the degree of inflammation (p=0.1). Inflammation was not associated with the degree of fibrosis (p=0.08).

According to these results and as illustrated in Table 4, we tried to define some patterns of NSP and their corresponding diagnostic efficacy in regards to underlying etiology (Table 5). Thus, a severely fibrotic pleura, with cellular fibrosis, vascular proliferation, hemorrhage, and neutrophils is more representative of bacterial disease (Pattern A). Lymphocyte-predominant inflammation with mild/moderate old collagenous fibrosis, with no layering or vascular proliferation, is mostly associated with viral disease (Pattern B). Moderate old collagenous fibrosis with variable degree of lymphocytic inflammation without vascular proliferation, edema, fibrin, hemorrhage or mesothelial hyperplasia is seen with heart disease (Pattern C). Autoimmune disease presents with

important cellular, layered fibrosis with moderate lymphocytic inflammation, as well as vascular proliferation, oedema and fibrin without hemorrhage (Pattern D). A similar pattern but with hemorrhage and without vascular proliferation is seen in drug-induced cases (Pattern E). In contrast to these etiologies, pleural tissue associated with recurrent pneumothorax is characterized by numerous histiocytes and eosinophils and by mild, if any, collagenous fibrosis (Pattern F). These patterns were significantly associated with the etiology ($p<0.0001$) and showed interesting values of diagnostic efficacy for each pattern (Table 5).

Discussion

Non-specific pleuritis is the term usually applied to a pleural effusion after biopsy has excluded a “specific” and often definitive diagnosis, mostly malignancy or granulomatous disease (7). In this case, a descriptive diagnosis is given usually reporting features like inflammation or fibrosis (7). The etiology hidden behind this histology is variable and impossible to define without the complete clinical data. We show in this study that the various diseases presenting with a non-specific pattern in pleural biopsy differ in their detailed histological features.

In particular, bacterial diseases present predominantly with a severely fibrotic pleura and this fibrosis is cellular characteristically rich in fibroblasts. Vascular proliferation in the form of rich granulation tissue as well as hemorrhage and tissue layering are also frequent features of these infectious conditions. Furthermore, neutrophils as the predominant cellular type in pleural biopsy are almost exclusively seen with this etiological group. Indeed this pattern (A of our classification) proved to have a high positive predictive value and therefore is highly specific for bacterial infection (Table 5). Pleural effusions associated with viral infections are lymphocyte-predominant in histological specimens, showing mild or moderate fibrosis, old collagenous in type and no layering of the tissue or vascular

proliferation. This proposed pattern (Pattern B of our classification) presents high negative predictive value and sensitivity (Table 5). Pleural effusion associated with heart disease shows variable degree of inflammation but always lymphocyte-predominant and moderate fibrosis, which is mostly old collagenous without vascular proliferation, edema, fibrin, hemorrhage or mesothelial hyperplasia. The proposed pattern C in our classification showed relatively high specificity (Table 5). Autoimmune disease presents with a moderate lymphocyte-predominant inflammation, important fibrosis cellular in type and layered, as well as vascular proliferation, edema and fibrin without hemorrhage. This pattern (Pattern D) showed high negative predictive value and sensitivity (Table 5). A similar pattern but with hemorrhage and without vascular proliferation is seen in drug-induced cases. In this case, the proposed pattern E presents high specificity (Table 5). In contrast to these etiologies, pleural tissue associated with recurrent pneumothorax is characterized by an inflammation rich in histiocytes and eosinophils and by mild, if any, fibrosis that is old collagenous in type. Interestingly, this pattern classified as F proved to present the highest sensitivity and specificity for diagnosis of pneumothorax (Table 5).

These differences in histological findings provide a pattern of NSP varying according to the underlying etiology and the pathophysiology of pleural effusions. Histological changes found in the current effusions series reflect the underlying tissue remodeling seen during pleural injuries. During pleural injury, increased microvascular permeability, induces plasma extravasation and activation of tissue factor and thus initiation of coagulation and formation of fibrin (11). This disordered local fibrin turnover represents an early tissue response subject to the influence of local mediators of inflammation and of components of the fibrinolytic response and sets the stage for progressive remodeling/wound healing or fibrotic repair in the face of protracted injury (11). TGF-b as the major pro-fibrotic factor, and subpleural fibroblasts but also mesothelial cells

that can undergo epithelial to mesenchymal (EMT)-like transition as the main cell targets, are the basic players in pleural fibrosis (11). According to our findings, bacterial and autoimmune diseases will show the most prominent remodeling of the pleural tissue probably reflecting an imbalance in favor of the coagulation and opposing to the fibrinolytic response, while viral and cardiac etiologies represent a more balanced response with less tissue remodeling. Chronic air trauma/local bleeding due to adhesions rupture in recurrent pneumothorax does not evoke an important tissue remodeling or imbalance of the fibrinolytic/coagulation system, rather evokes a foreign body reaction with eosinophils and histiocytes overloading the pleural surface.

As from a clinical point of view, NSP often presents a management dilemma for treating physician as it could represent a true benign etiology or just a sampling/diagnostic error that would then imply the need for further invasive techniques. Studies trying to answer to the question of evolution of these non-specific pleuritis cases after long-term follow-up showed that in 5% (6) to 18% (12, 13) of the cases a malignancy, particularly mesothelioma, will be revealed and thus a minimum of 1 year of clinical and imaging follow-up is recommended (8) (14) (15), as was the case in our series. Also, important criteria to facilitate the diagnosis of underlying malignancy in case of NSP are past or active history of carcinoma (16). These false-negative cases represent the difficulties in sampling a thickened pleura not allowing deep biopsies to be easily performed or a pleural cavity full of adhesions difficult to inspect (5). The endoscopic appearance of pleural lesions is suggestive of malignancy in 86% of cases and suspicious appearances include nodules, polypoid lesions, masses, malignant thickening of the pleura, and localized “candle wax drops” (17). However, benign conditions can be mistaken for malignant ones and vice versa (17). False-negative diagnoses could also represent cases difficult to handle histologically as an intense fibrosing pleuritis may difficult to discern from a

sarcomatoid or desmoplastic mesothelioma (18). We have recently shown that frozen sections in pleural disease are very efficient in rapidly diagnosing malignancy (19) and for tissue handling for molecular tests (20).

Similar with NSP cases, eosinophilic pleural effusions which are defined as effusions with 10% or more eosinophils, can be attributed to many causes (air or blood in the pleural cavity, malignancies, drugs, asbestos exposure, infections, or autoimmune diseases) or they can be idiopathic after exclusion of all these known etiologies (21) (22). Long-term follow-up of these “idiopathic” cases has revealed a benign clinical course but this can be reassured only after thorough thoracoscopic investigation of the pleural cavity and benign corresponding histology (21).

To conclude, our study provides the first evidence for the microscopic procedures underlying benign pleural diseases, and could lead to further investigation regarding the mechanisms driving the course of the disease. Our classification may also be applied in the routine practice by adding a comment on the suggesting pattern in the pathology report addressed to the clinician, which can be helpful in the final diagnosis of the underlying pleural disease. Indeed, although in our series all patients were found to present a benign disease, significant microscopic differences were observed reflecting the capacity for tissue remodeling in every case. It also opens new horizons for studying pleural inflammation to establish mechanisms in the pathogenesis of pleural involvement according to the underlying disease.

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Authorship contribution: MEF conceived, designed the study, analyzed data, drafted and corrected the manuscript – GK designed the study, reviewed all pleural biopsies, drafted the manuscript – SH, FC, CB collected patients' data – FF, MP designed the study, reviewed biopsies – AP, OT, CB, MF collected thoracoscopy data – NMR drafted and corrected the manuscript. All authors reviewed and approved the final draft.

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Table 1. Patients characteristics (*n=100*)

	n, (%)
Age (years)	
Median	63
Range	13-94
Sex	
Male	71 (71)
Female	29 (29)
Follow up (months)	
Median	36
Range	1-48
Final aetiology	
- Pneumothorax	28 (28)
- Bacterial Infection	27 (27)
- Cardiac	10 (10)
- Drug induced	7 (7)
- Viral Infection	7 (7)
- Auto-immune disease	5 (5)
- Other	16 (16%)
Surgery-trauma	2
Pleural plaque	2
Dressler	1
Kidney failure	1
Pulmonary embolism	1
Radiotherapy	1
Chylothorax	1
Eosinophilic	1
Unkown-idiopathic	6
Localization	
Left	44 (44)
Right	42 (42)
Bilateral	11 (11)
Predominant pleural aspect during thoracoscopy	
Inflammatory	40 (40)
Fibrinous	14 (14)
Fibrous	6 (6)
Septated	8 (8)
Hemorragic	2 (2)
Pleural plaques	2 (2)

Table 2. Histological evaluation in all patients except "Other" group *(n=84)*

Parameters	n (%)
Inflammation	
No	7 (8,4)
Mild	23 (27.7)
Moderate	41 (49.3)
Severe	12 (14.4)
Predominant inflammatory cell type (*n=76*)	
Lymphocytes	49 (64.4)
Plasmocytes	10 (13.1)
Neutrophils	9 (11.8)
Histiocytes	7 (9.2)
Eosinophils	1 (1.3)
Fibrosis	
No	7 (8.4)
Mild	30 (36.1)
Moderate	19 (22.8)
Severe	27 (32.5)
Type of fibrosis (*n=76*)	
Cellular	29 (38.1)
Old	39 (51.3)
Both	8 (10.5)
Layering	
Yes	28 (33.8)
No	55 (66.2)
Vascular proliferation	
Yes (score 2 and 3)	41 (49.3)
No (score 0 and 1)	42 (50.6)
Oedema	
Yes (score 2 and 3)	17 (20.4)
No (score 0 and 1)	66 (79.5)
Haemorrhage	
Yes (score 2 and 3)	19 (23.1)
No (score 0 and 1)	63 (76.8)
Fibrin	
Yes (score 2 and 3)	34 (40.9)
No (score 0 and 1)	49 (59.1)
Mesothelial Hyperplasia	
Yes (score 2 and 3)	11 (13.2)
No (score 0 and 1)	72 (86.7)
Number of slides examined per case	
Median (range)	4 (1-18)
Specimen size (cm)	
Median (range)	7 (1-28)
Histiocytes	
Yes	24 (28.8)
No	59 (71.2)

Table 3. Clinicopathologic correlations

	Bacterial n=27	Viral n=7	Cardiac n=10	Pneumo-thorax n=28	Auto-immune n=5	Drug n=6	p
Final score (mean)	5.1±1.4	4.4±1.6	3.8±1.2	3.1±1.5	5.6±1.1	4±1.4	**<0.0001**
Inflammation							
No	3	1	0	3	0	0	
Mild	6	0	3	11	0	3	0.3
Moderate	12	4	5	13	5	2	
Severe	6	2	2	1	0	0	
Predominant inflammatory cell type							
Lymphocytes	14	7	9	11	5	4	
Plasmocytes	3	0	1	5	0	1	**0.02**
Neutrophiles	7	0	0	1	0	1	
Histiocytes	0	0	0	7	0	0	
Eosinophiles	0	0	0	1	0	0	
Fibrosis							
No	2	0	2	2	0	1	
Mild	3	3	3	18	0	2	**0.03**
Moderate	6	2	2	6	2	1	
Severe	15	2	3	2	3	2	
Type of fibrosis							
Cellular	17	1	3	5	1	2	
Old	5	5	4	20	2	3	**0.006**
Both	3	1	1	1	2	0	
Layering							
Yes	13	1	4	4	3	3	**0.05**
No	14	6	6	24	2	3	
Vascular proliferation							
Yes (score 2 and 3)	24	3	3	7	3	1	**<0.0001**
No (score 0 and 1)	3	4	7	21	2	5	
Oedema							
Yes (score 2 and 3)	7	1	0	4	2	3	0.1
No (score 0 and 1)	20	6	10	24	3	3	
Fibrin							
Yes (score 2 and 3)	16	2	3	7	3	3	0.1
No (score 0 and 1)	11	5	7	21	2	3	
Heamorrhage							
Yes (score 2 and 3)	12	1	2	2	0	2	**0.02**
No (score 0 and 1)	15	6	8	25	5	4	
Presence of histiocytes							
Yes	0	3	1	20	0	0	**<0.0001**
No	27	4	9	8	5	6	
Mesothelial hyperplasia							
Yes	0	1	0	9	0	1	
No	27	6	10	19	5	5	**0.009**

Table 4. Histological features according to etiology

	Bacterial	Viral	Cardiac	Pneumothorax	Autoimmune	Drug
Inflammation	++/+++	++/+++	+/++	+/++	++	+/++
Lymphocytes	++	++	++	++	++	++
Plasmacytes	+	-	-	+	-	-
Neutrophils	++	-	-	-	-	-
Histiocytes	-	-	-	++	-	-
Eosinophils	-	-	-	++	-	-
Fibrosis	+++	+/++	++	+/-	+++	+/-
Cellular fibrosis	+++	+/-	+/-	+/-	+/-	+/-
Layering	+++	+/-	+	+/-	+++	+++
Vascular proliferation	+++	++	+	+/-	+	+/-
Oedema	+	+/-	-	+/-	+/-	+
Fibrin	+++	+/-	+/-	+/-	++	++
Haemorrhage	+++	+/-	+/-	+/-	-	+/-
Mesothelial hyperplasia	-	+/-	-	++	-	+/-

-: absent, +: mildly present, ++: moderately present, +++: usually present.

Table 5. Efficacy of the proposed patterns for dignosing the underlying cause

	Pattern A= Bacterial	Pattern B= Viral	Pattern C= Cardiac	Pattern D= Autoimmune	Pattern E= Drug	Pattern F= Pneumothorax
PPV	100%	35%	20%	21.74%	57.14%	90.32%
NPV	84.88%	100%	90.53%	100%	96.77%	100%
Sensitivity	51.85%	100%	10%	100%	57.14%	100%
Specificity	100%	86.02%	95.56%	81.05%	96.77%	95.83%

PPV: positive predictive value
NPV: negative predictive value

Figures legends

Figure 1. Fibrosis histological assessment. A/ A normal parietal pleura biopsy showing mesothelial cells and the underlying connective tissue with its vascular elements (HES x 200). **B/** Mild collagenous fibrosis overlying parietal pleura adipose tissue and mild lymphocytic inflammation of a heart-disease associated NSP (HES x 50). **C/** Moderate collagenous fibrosis overlying parietal pleura adipose tissue in a case of a heart-disease associated NSP (HES x 40). **D/** Severe cellular fibrosis replacing the whole pleural membrane accompanied by hemorrhage on the cavity surface in a bacterial NSP (HES x 20).

Figure 2. Stages during inflammation/wound healing in bacterial-caused NSP. **A/** A biopsy showing prominent tissue layering: the initial pleural membrane with overlying inflammation (blue line), edema (red line) and vascular-rich organization tissue (black line)(HES x 20). **B/** Another case of tissue organization, showing conspicuous vascular proliferation inside a fibrotic and inflammatory stroma (HES x 40). **C/** The typical morphology of a fibroblast-rich fibrosis with layering of cells (HES x 200). **D/** For comparison, a case of viral-induced NSP showing old collagenous fibrosis at the bottom with a mildly cellular fibrosis at more superficial levels (mixed fibrosis, HES x 100).

Figure 3. Representative patterns of NSP. A/ Bacteria-associated NSP showing a thickened layered fibrotic pleura with rich cellular fibrosis, inflammation and hemorrhage at the surface (HES x 40). **B/** A viral-associated NSP showing moderate chronic inflammation, mild mesothelial hyperplasia and no fibrosis (HES x 100). **C/** A cardiac NSP showing moderate old fibrosis and mild inflammation (HES x 40). **D/** A pneumothorax-associated NSP showing a mild old collagenous fibrosis and a rich histiocytic infiltrate often with giant cells and eosinophiles leucocytes (HES x 100).

HES: Hematoxylin, Eosin, Saffran
NSP: Non-specific pleuritis