

Aus dem Institut für Pathologie
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DISSERTATION

**Immunohistochemistry in diagnostic pathology of tumors.
Approach, benefits, limits and pitfalls**

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1. Introduction

Histopathological tumor diagnosis and tumor classification in addition to the identification of the histogenesis of metastases of unknown or uncertain primary tumors are considered to be the most important responsibilities of practical histopathologists. At the present time, in addition to the traditional light microscopy, there is a list of other informative methods that support histopathologists in their work such as electron microscopy, histochemistry, immunohistochemistry and different molecular methods. In the past 20 years, immunohistochemistry was dramatically developed and became a very powerful and simple tool in diagnostic histopathology. Many steps of immune-stain protocols were markedly simplified and a large number of diagnostic antibodies were introduced to resolve many diagnostic problems and to increase the diagnostic certainty. Nevertheless immunohistochemistry - as any method - has its own possibilities and limitations and every pathologist must be aware of the diagnostic pitfalls that may occur practicing this method. In this study, we are going to emphasize the role of immunohistochemistry in diagnostic tumor histopathology and to analyze the most common diagnostic mistakes and pitfalls.

2. Aim of the study

Nowadays, immunohistochemistry is a widely used sensitive method in histopathology. It is essential to differentiate the phenotype of normal and neoplastic cells and to detect tumor specific antigens using a large panel of highly specific monoclonal and polyclonal antibodies specific for the majority of cell lines at different stages of differentiation.

In the past 5 years, we at the German-Jordan Center for Laboratory Medicine performed about 18000 immunohistochemical stains mainly for tumor diagnosis as a reference laboratory for immunohistochemistry and molecular pathology. Based on the results and experiences obtained using more than 110 different antibodies on a wide spectrum of tissue and tumor types, we started this study with the following aims:

- I. To highlight the benefits and possibilities of immunohistochemistry as a powerful diagnostic tool in tumor histopathology essential for tumor diagnosis, tumor classification and tumor follow up.
- II. To establish a rational approach for diagnostic immunohistochemistry in tumor histopathology. The optimal approach must be time, labor and money saving, based on excellent professional knowledge concerning the method, biology and expression spectrum of targeted antigen and specifications of used antibodies and all of this must be crowned by an informative standardized and logical way of result interpretation and documentation.
- III. To clarify the border line between the benefits and limitations of immunohistochemistry in tumor histopathology, as every pathologist must be aware of the limits of the used method and when to switch to another complementary method.
- IV. Another important aim of this study is to examine and explain the sources of and reasons for diagnostic pitfalls in diagnostic immunohistochemistry
- V. A last important aim was to develop suggestions and recommendations to increase the value of immunohistochemistry as an informative diagnostic method in tumor histopathology and to minimize the incidence of diagnostic mistakes.

3. Material and methods

3.1. Material

In the time between 2004-2009, we at the German Jordan Center for Laboratory Medicine performed about 18000 immunohistochemical stains using more than 110 primary antibodies on sections obtained from formalin-fixed-paraffin embedded human tissue. The specimens came to us directly from the regional laboratories and hospitals or from the regional oncologists. The Patients were Jordanians or from one of the following neighbor countries including Syria, Iraq, Sudan Yemen and Libyan. The patients were between 8 months and 92 years old. 2907 tumors in 3720 paraffin blocks were examined. The tissue blocks were prepared by us or by other laboratories or were brought by the patients from their home countries. The tumors were from different locations as listed below:

643 Lymph nodes with primary or secondary neoplasia

387 Breast tumors

331 Upper and lower respiratory tract specimens with primary or secondary tumors

254 Gastrointestinal tract biopsies

195 Prostatic biopsies

174 Bone trephines for hematological malignancies and tumor staging

108 Soft tissue biopsies with primary or secondary tumors

85 Skin tumors

79 Liver core biopsies or surgical specimens with primary or secondary tumors

76 Primary and secondary intracranial and brain tumors

63 Pleural biopsies with primary or secondary tumors

57 Primary and secondary peritoneal tumors

54 Ovarian tumors

49 Mediastinal biopsies

46 Renal tumors

38 Uterine tumors

36 Testicular tumors

33 Thyroid tumors

31 Tumors of uterine cervix

- 30 Urinary bladder and urethral tumors
- 30 Bone biopsies with primary or secondary tumors
- 25 Retroperitoneal tumors
- 24 Oral tumors
- 19 Suprarenal gland specimens with pediatric or adult tumors
- 18 Tumors of salivary glands
- 12 Pancreatic tumors
- 06 Ocular or periocular tumors
- 04 Parathyroid tumors

The immunohistochemical study was done to resolve one or more of the following diagnostic issues:

- To determine the histogenesis of primary neoplasia (carcinoma, lymphoma, hematological neoplasia, soft tissue tumor etc) followed by the classification of the tumor type (type of carcinoma, type of lymphoma, type of soft tissue tumor etc).
- To determine the histogenesis of metastatic tumor of unknown origin.
- To determine the criteria of malignancy in doubtful lesions. Prostatic core biopsies to label the myoepithelial cells and breast biopsies to prove the presence of carcinoma in situ or microinvasion were the most common subject for this examination.
- To confirm the diagnosis as second opinion prior to tumor therapy. The majority of tumor blocks were brought by the patients from their home countries. In many cases the examined tissue was suboptimal due to poor fixation or bad processing.
- To determine the sensitivity of tumors to related anti-cancer drugs including steroid receptors, HER-2 oncoprotein, epidermal growth factor receptor and CD117 (c-kit).

3.2. Used antibodies

1. Actin 1 (clone: 1A4)
2. Alfa Fetoprotein (clone: C3)
3. ALK (ALK1)
4. AMACR (poly)
5. Androgen receptors (clone: AR27)
6. BCL2 (clone: CL.124)
7. BCL-6 (clone: PG-B6P)
8. β -HCG (poly)
9. CA125 (clone: M11)
10. CA19-9 (clone: 1116 NS 19-9)
11. Calcitonin (clone: Cal3-F5)
12. Caldesmon (clone: h-CD)
13. Calponin (clone: 26A11)
14. Calretinin (clone: 5A5)
15. D2-40 (clone: D2-40)
16. CD 1a (clone: O10)
17. CD 2 (clone: AB75)
18. CD 3 (clone: F7.2.38)
19. CD 4 (clone:4B12)
20. CD 5 (clone: 4C7)
21. CD 7 (clone: OV-TL)
22. CD 8 (clone: 1A5)
23. CD 10 (clone: 56C6)
24. CD 15 (clone: C3D-1)
25. CD 19 (clone: 2E2B6B10)
26. CD 20 (clone: L26)
27. CD 21 (clone: 2G9)
28. CD 30 (clone: Ber-H2)
29. CD 31 (clone: JC/70)
30. CD 34 (clone: QBend 10)
31. CD38 (clone: LCD38-290)
32. CD 43 (clone: MT1)
33. CD45 (LCA)(clone: 2B11+PD7/26)
34. CD 56 (clone: CD56-504)
35. CD 61 (clone: Y2/51)
36. CD 68 (clone: PG-M1)
37. CD 79a (clone: 11E3)
38. CD 99 (clone: 12E/7)
39. CD 117 (poly)
40. CD 138 (clone: MI15)
41. CD 141 (clone:1009)
42. CDX-2 (clone: AMT 28)
43. CEA (clone: II-7)
44. Chromogranin (clone: DAK-A3)
45. CK 5/6 (clone: D5/16 B4)
46. CK 5/14 (clone: XM26/LL002)
47. CK 7 (clone: OVTL)
48. CK 8/18 (clone: 5D3)
49. CK 18 (clone: Ks18.4)
50. CK 19 (clone: RCK 108)
51. CK 20 (clone: K_s20.8)
52. CK-HMW (clone: 34bE12)
53. CK-MNF (clone: MNF116)
54. CK-Pan (clone: LP34)
55. Cyclin D1 (clone: DCS-6)
56. Desmin (clone: D33)
57. Dog 1 (clone: sp31)
58. E-Cadherin (clone: NCH-38)
59. EGFR-1 (clone:31G7)
60. EMA (clone: E29)

61. Estrogen (clone: 1D5)
62. F VIII (poly)
63. Fascin (clone: 55K-2)
64. FLI-1 (poly)
65. GCDFP-15 (clone: 23A3)
66. GFAP (clone: 6F2)
67. Hepatocyte (clone: OCH1E5)
68. Her-2 (poly)
69. HHV-8 (clone: 13B10)
70. HMB45 (clone: HMB45)
71. Inhibin A (clone: R1)
72. Kappa (clone: R10-2L F3)
73. Ki-67 (clone: MIB-1)
74. Lambda (clone: N10 2)
75. Mast cell tryptase (clone: AA1)
76. Mammaglobin (poly)
77. MDM2 (clone: SMP14)
78. Melan A (A103/M2-7C10/M2-9E3)
79. Mesothelin (clone: 5B2)
80. MPO (poly)
81. MUM1 (clone: mum-1p)
82. Myelin basic protein (clone: 7H11)
83. MyoD1 (clone: 5.8A)
84. Myogenin (clone: F5D)
85. Myoglobin (clone MYO18)
86. Myosin (clone: Y32)
87. Neuroblastoma (clone: NB84a)
88. NF (clone: 2F11)
89. NSE (clone: BBS/NC/VI-38)
90. Oct-3/4 (clone: N1NK)
91. P16 (clone: INK4)
92. P53 (clone: PAb240)
93. P63 (clone: 4A4)
94. Pan-melanoma (HMB45, MART 1 -A103, Tyrosinase- T311)
95. PAX-5 (poly)
96. PGP 9.5 (poly)
97. Placental alkaline phosphatase (clone: 8A4)
98. Plasma cell (clone: VS38c)
99. Podoplanin (clone: D2-40)
100. Progesterone (clone: PgR636)
101. Prostate specific antigen (clone:ER-PR8)
102. Renal cell carcinoma (clone: gp200)
103. S100 (poly)
104. Surfactant protein A (clone: 32E12)
105. Synaptophysin (clone: SY38)
106. TDT (poly)
107. Thyroglobulin (clone: RBU/01)
108. TTF-1 (clone: 8G7G3/1)
109. Vimentin (clone: V9)
110. WT1 (clone: 6F-H2)

3.3. Methods

All immunohistochemical stains were performed on tissue sections prepared from formalin-fixed paraffin-embedded tissue blocks. According to sample dimensions, 10-24 hours fixation time in 10% buffered formalin was generally achieved. We also found that the use of buffered zinc formalin gives superior results and for many antibodies the antigen retrieval step is not required, which is of benefit in the cases of small tiny tissue fragments. After the unmasking of the tissue antigens, the sections were incubated with the primary antibodies. The antibody-antigen complex was labeled using the streptavidin-biotin horseradish peroxidase detection system or the polymer detection system.

3.3.1. Immunohistochemical staining

Immunohistochemical staining protocol

In our work, the following manual staining protocol was performed:²

1. 2-3 μm thin paraffin sections were cut and transferred on electrostatically charged or silane-coated slides, dried overnight at 37°C or incubated one to two hours at 55°C. In urgent cases, slides were dried in a microwave oven for 3-4 minutes on high power.
2. Sections were deparaffinized and hydrated to H₂O: 30 minutes xylene - 2 minutes absolute ethanol - 2 minutes 96% ethanol - 2 minutes 70% ethanol - 2 minutes 40% ethanol - H₂O.
3. Antigen unmasking: Two main methods were used to restore the original structure of antigens (unmasking) so as to be recognized by the primary antibody:¹
 - A. Heat-induced unmasking: This method was used for the majority of antibodies. We used a pressure cooker for microwave heating. The buffers used have different pH, usually ranging between 6 and 9. The citrate-based buffer (pH 6-7) was the most commonly used buffer; other buffers such as EDTA or Tris-based buffers with higher pH (8-9.9) were used for very few antibodies. A further benefit of using the heat-induced unmasking method is to decrease the reactivity of endogenous biotin maybe present in the processed tissue, which may cause some artifacts or nonspecific background.
 - B. Enzymatic digestion: This method was used for very few antibodies. The hydrated slides were incubated in phosphate buffered saline (PBS), pH7.2 for 5 minutes and then covered by pronase

solution (0.05 % working concentration) for 10-15 minutes at 37°C in humid chamber. Protease with 0.1% working solution was also used. After the enzymatic digestion, the slides were carefully washed in PBS for 5 minutes.

4. Blocking of endogenous peroxidase: The slides were incubated for 10 minutes at room temperature in a jar filled with 0.3% hydrogen peroxide (H₂O₂) solution and then washed in PBS for 2-3 minutes.
5. To reduce any nonspecific reaction, 100 µl of non-immune serum was added to the slides and incubated for 15 minutes.
6. 50-100 µl of the primary antibody with adequate concentration was added and incubated for 30-60 minutes at 25-37°C.
7. The slides were washed with PBS 3 times, 2-3 minutes each.
8. 50-100 µl of biotinylated secondary antibody was added and incubated for 15-30 minutes at 25-37°C.
9. The slides were washed with PBS 3 times, 2-3 minutes each.
10. 50-100 µl of the avidin / biotinylated horseradish peroxidase complex (ABC) was added and incubated for 15-30 minutes at 25-37°C.
11. If the polymer detection system was used, steps 8, 9 and 10 were replaced by adding 50-100 µl of the polymer detection system.
12. The slides were washed with PBS 3 times, 2-3 minutes each.
13. 100 µl of pre-warmed (~25°C) DAB (3, 3' diaminobenzidine tetra-hydrochloride, C₁₂H₁₄N_{4.4}HCl) chromogen solution was added and incubated for 5-8 minutes. The DAB chromogen produces a brown hydrophobic end product, resistant to alcohol and xylene.
14. To increase the intensity of the stain (in some cases) slides were incubated in 1-5% copper sulfate solution. This treatment produces dark brown stain.
15. The slides were washed with H₂O and stained with hematoxylin for 4-8 seconds and washed with H₂O for 5 minutes. Slides can be also mounted without counterstaining.
16. Slides were dehydrated with 40% - 70% - 96% - 100% ethanol and xylene and finally mounted using xylene-based mounting media.

3.3.2. Immunohistochemical double staining

The immunohistochemical double stain with two antibodies was performed in some cases to improve the stain results or to differentiate between two different cell types in the same slide. The following approach was considered in the immunohistochemical double stain:

I. Staining of two different cell types

Suitable for this purpose are antibodies reacting with antigens with nuclear expression pattern (PAX-5, P63, TTF-1 or other transcription factors) in combination with antibodies reacting with antigens with membranous or cytoplasmic expression pattern.

- a. **Pin cocktail:** the PIN cocktail is a combination of the P63 protein and P504S (α-methylacyl-CoA racemase, AMACR). P63 is expressed in the nucleus of basal cells of benign prostatic glands in addition to the basal cells of other benign glandular structures of other organs. P505S is highly expressed in prostatic adenocarcinoma, in prostatic premalignant lesions including high grade prostatic intraepithelial neoplasia (PIN) and atypical adenomatous hyperplasia but usually not in benign prostatic glands. The use of this antibody cocktail is very helpful for the diagnosis of small foci of adenocarcinoma or low grade adenocarcinoma.
- b. **PAX- 5 and CD3 or CD2:** PAX 5 is a B- cell specific transcription factor. Specific antibodies to PAX-5 label B- lymphocytes at different maturation stages in addition of the majority of Reed-Sternberg cells with nuclear stain pattern. PAX-5 is not expressed in normal or neoplastic plasma- and T-cells. A combination of PAX-5 and T- cell markers such as CD2 or CD3 showing membranous stain will provide a good idea about the architecture of the lymphoid tissue.

II. Staining of cell cycle antigens and different histogenic markers

Ki-67 combined with other cell-specific antibodies with membranous or cytoplasmic stain. Ki-67 is a nuclear protein expressed in the G₁, S, G₂ and M phases of the cell cycle but absent only in the G₀ phase. The combination of Ki-67 with different lymphoid or other tissue specific markers will demonstrate the nature of active cells. This approach may be helpful for the diagnosis of lymphoid neoplasia and soft tissue tumors.

III. Staining to improve the sensitivity of the reaction

- a. Melanoma cocktail:** Since the available anti-melanoma antibodies do not have 100% sensitivity, a mixture of two or more melanoma specific antibodies such as HMB45, melan A, tyrosinase and PNL2 in one single reaction will be able to give higher sensitivity than a single antibody.
- b. Cytokeratin cocktails:** Different cytokeratins are expressed in different epithelial types at different stages of differentiation. A mixture of different antibodies reacting with type I and type II cytokeratins is very effective in detecting any epithelial differentiation. A mixture of different type II cytokeratins is also widely used to label the myoepithelial or basal cells. Known examples are cytokeratin 5/6 and cytokeratin 5/14 cocktails.
- c. Synaptophysin and Chromogranin:** Both synaptophysin and chromogranin are widely expressed in neural and neuroendocrine tissue. Since the expression of some neuroendocrine markers is minimal or absent in some neuroendocrine tumors, the use of a mixture of two or more neuroendocrine markers will increase the sensitivity to detect possible neuroendocrine differentiation. This approach will improve the diagnosis of neuroendocrine tumors including carcinoid and small cell carcinoma.

4. Immunohistochemical pathways for the diagnosis of metastasis of unknown primary tumors

Because of the large number of available antibodies, the choice of the primary screening antibody-panel must be done according to the morphology of the examined tumor, tumor location and clinical data, taking in consideration the specificity and the sensitivity of the used antibodies. For tumors with ambiguous morphology or tumors with undetermined histogenic differentiation, we found that the most informative, time- and money- saving primary panel consists of antibodies reacting with epithelial, mesenchymal, neural and hematopoietic cell lines as the following (figure 1):^{3, 4, 8}

1. Pan-cytokeratin (cytokeratin cocktail)
2. LCA (leukocyte common antigen)
3. S100 and HMB45 (or melanoma cocktail)
4. Vimentin

Other tissue specific markers can be added if the morphology of the tumors favors any differentiation line. For tumors with small round blue cell morphology, another screening antibody panel is necessary, which includes the following antibodies (figure 2):

1. S100
2. Pan-cytokeratin (cytokeratin cocktail)
3. Desmin and or myoglobin
4. LCA
5. CD99
6. CD30

This panel can be modified according to the age of the patient, tumor site and clinical history.

For orientation, we suggest a group of diagnostic algorithms to simplify solving the most common diagnostic problems (figures 3-9). According to the results obtained from these algorithms, a second panel with more selective antibodies can be assembled for final precise diagnosis.

In the following figures, general screening antibodies are placed in blue boxes, more specific antibodies in red boxes and diagnosis in green ones. It is always important to remember that the immunoprofile of tumors may be a subject of exceptions.

4.1. Diagnostic algorithms for tumor screening

Figure 1: Primary screening antibody panel

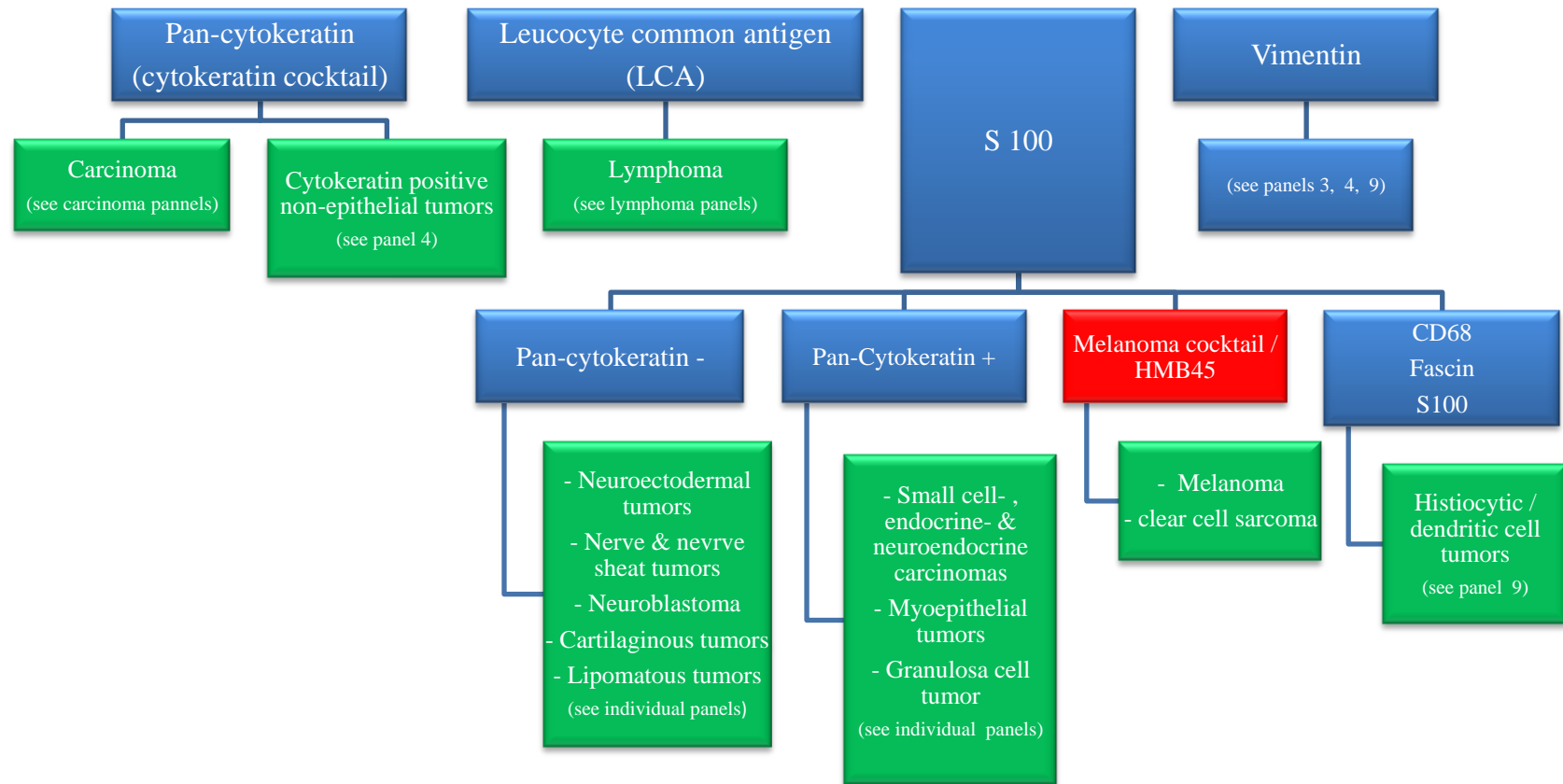


Figure 2: Antibody panel for tumors with small round blue cell morphology

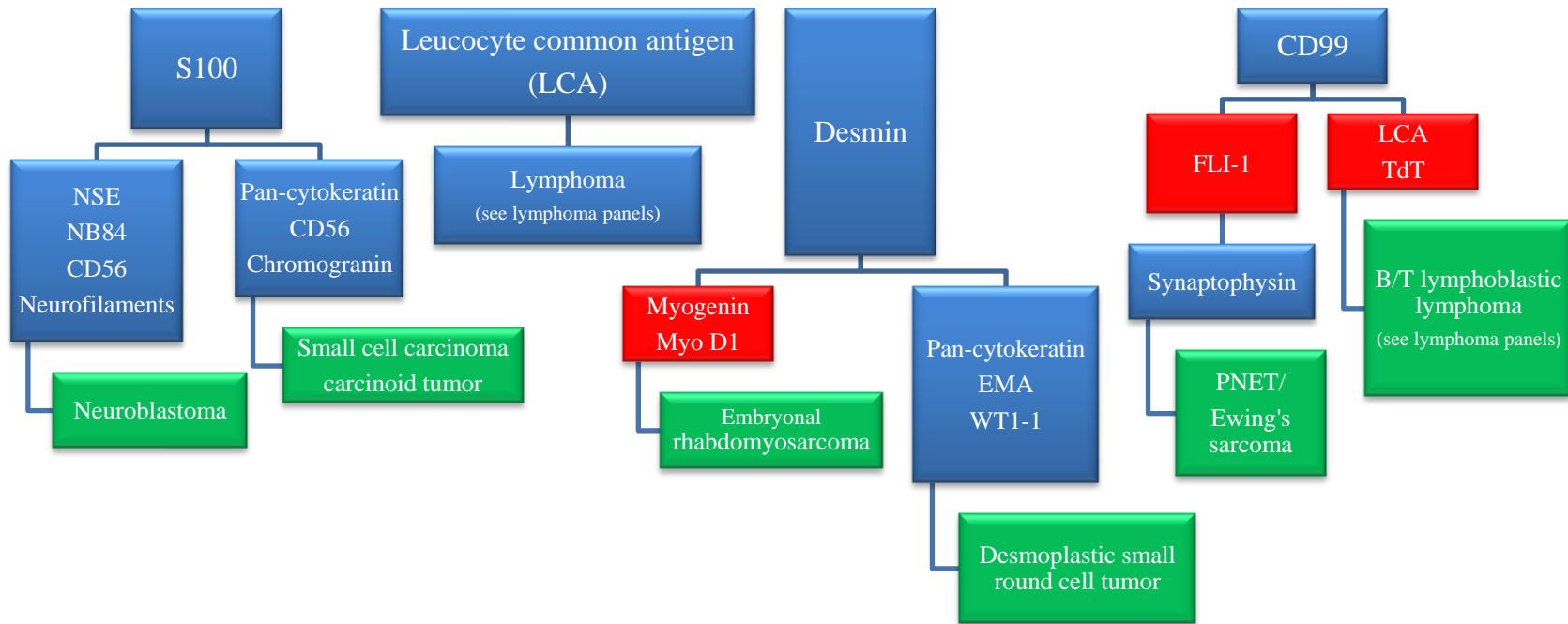


Figure 3: Algorithm for cytokeratin negative tumors

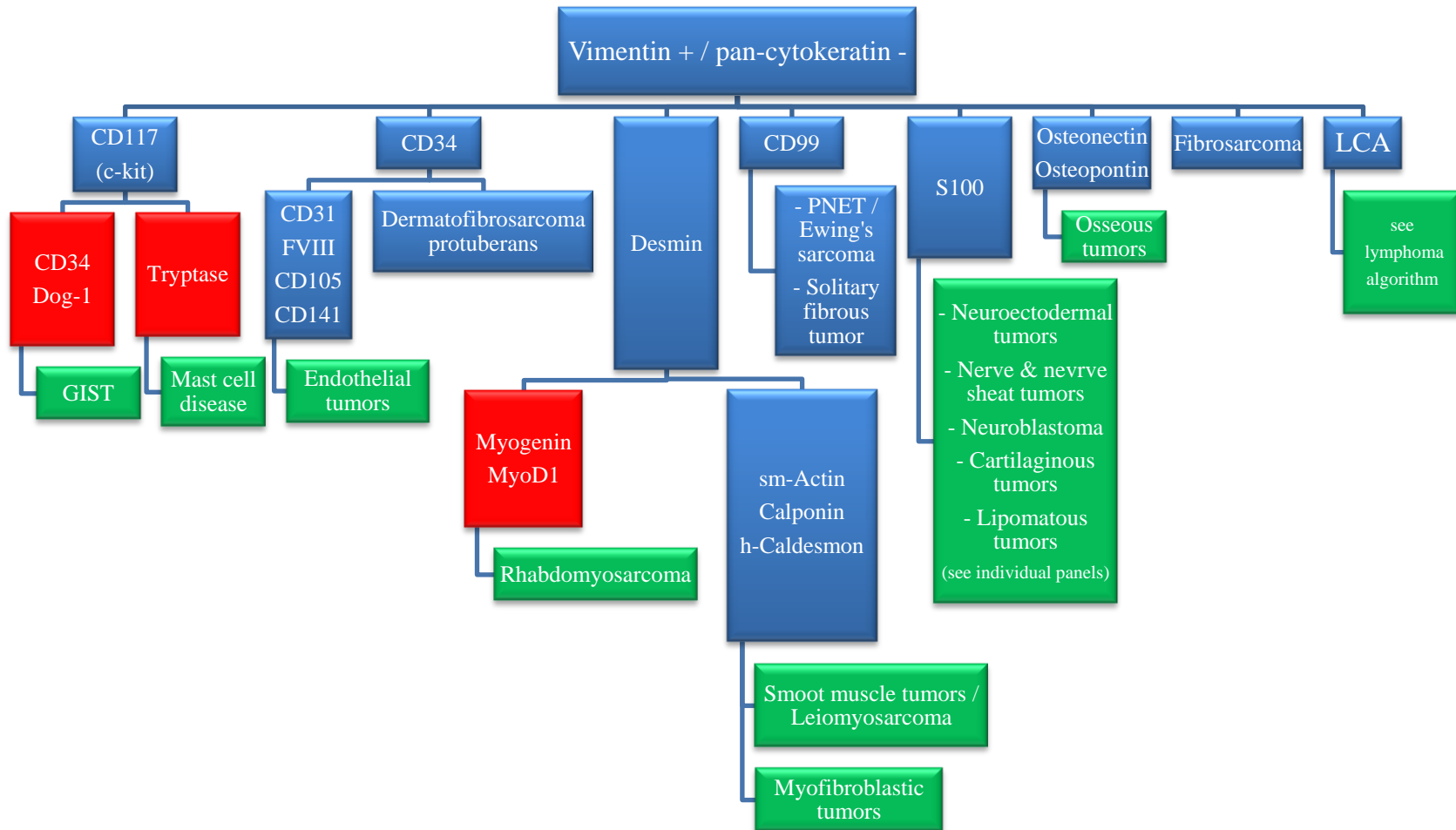


Figure 4: Algorithm for tumors with cytokeratin / vimentin co-expression

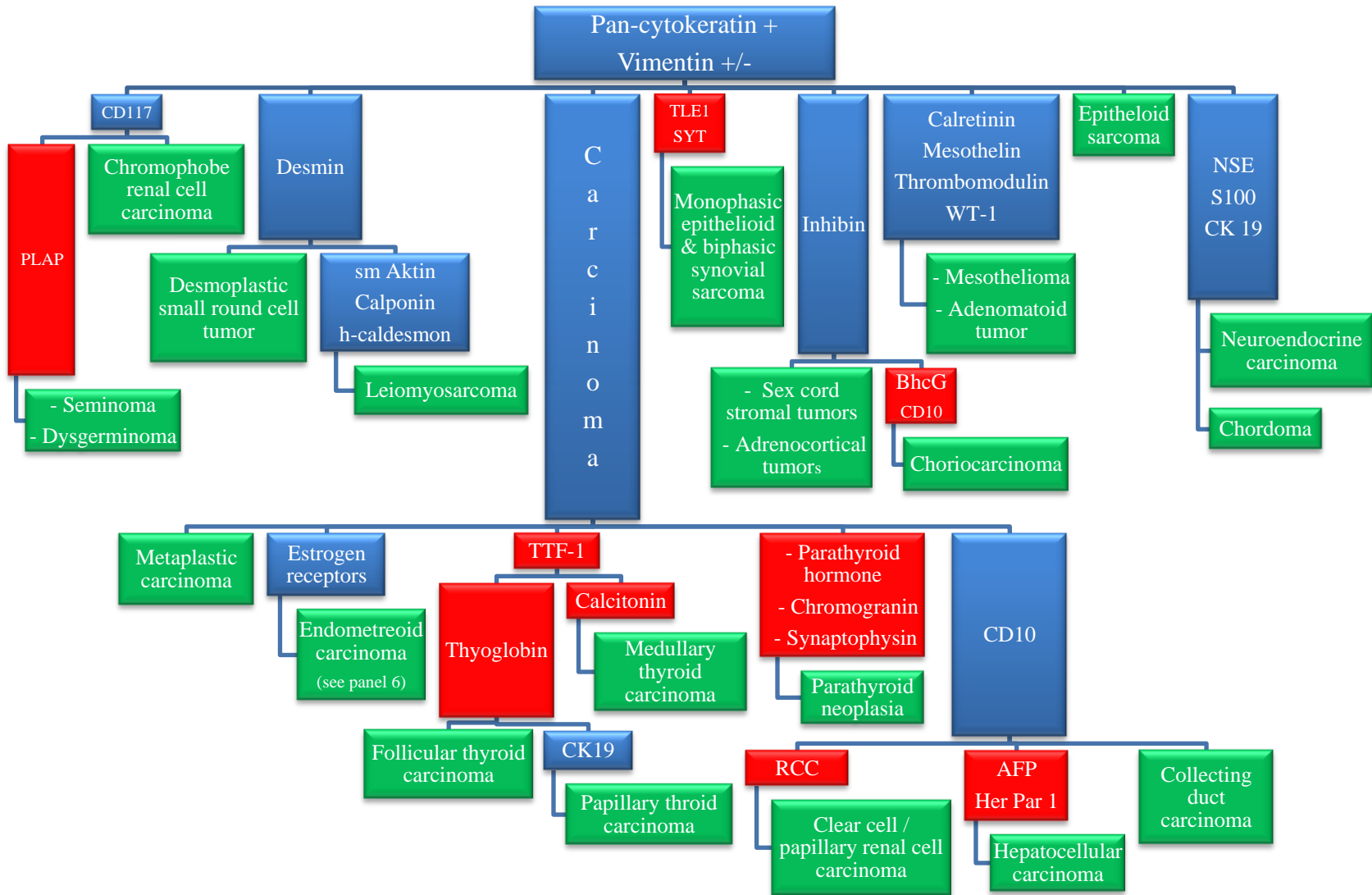


Figure 5: CK 7 / CK 20 algorithm

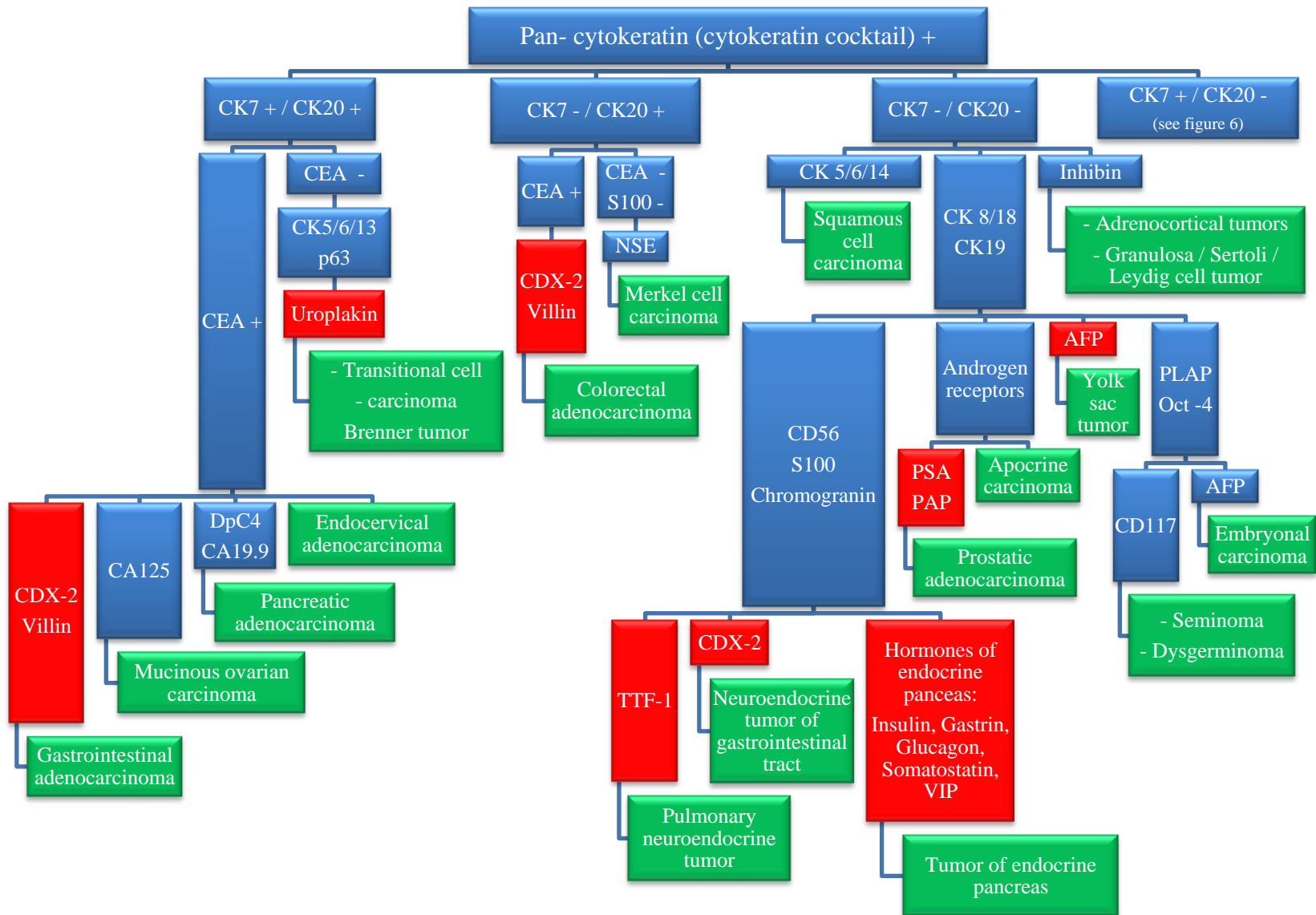


Figure 6: Algorithm for cytokeratin CK 7+ / CK20 - carcinoma

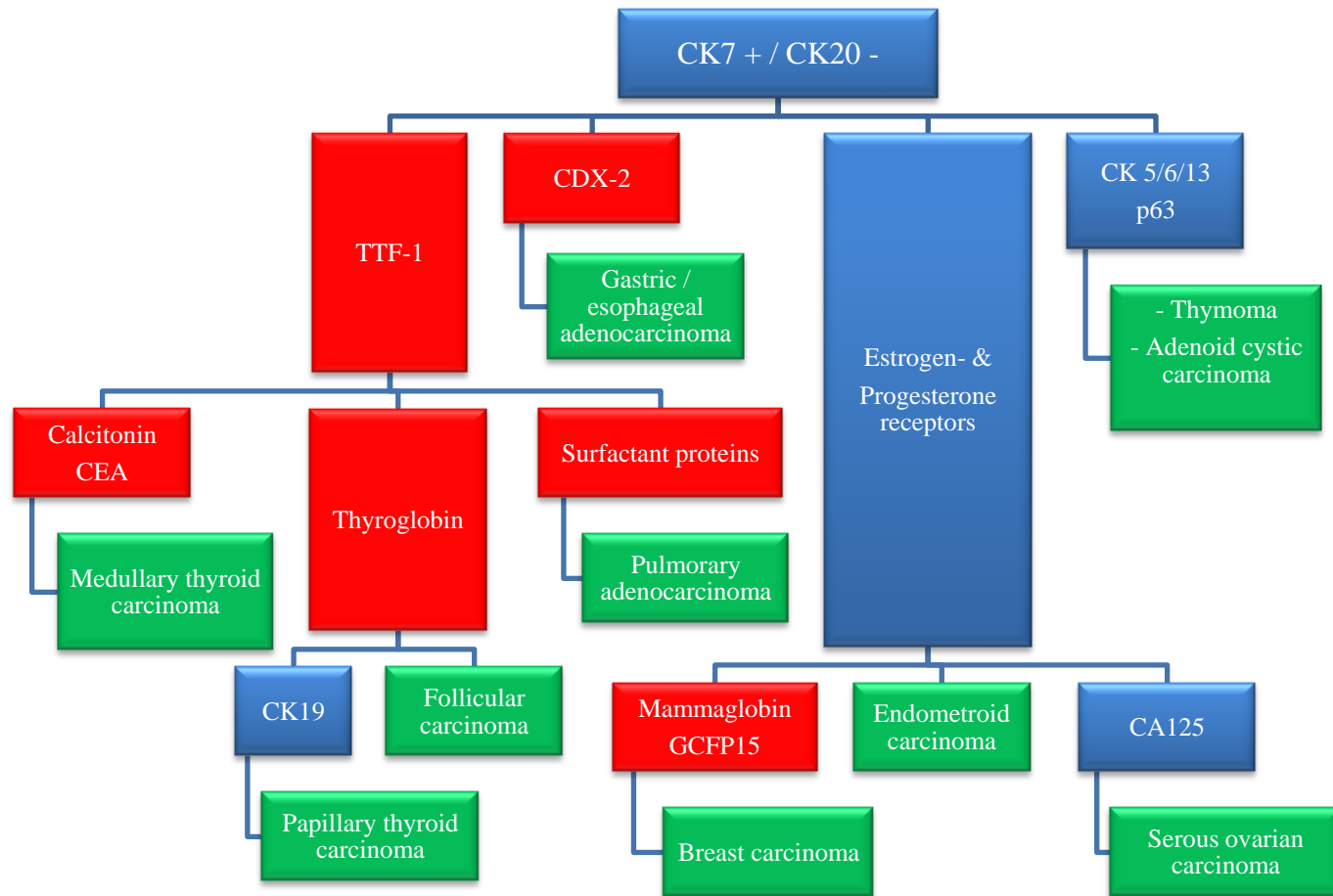


Figure 7: Algorithm for lymphoma, T/NK- cell neoplasia

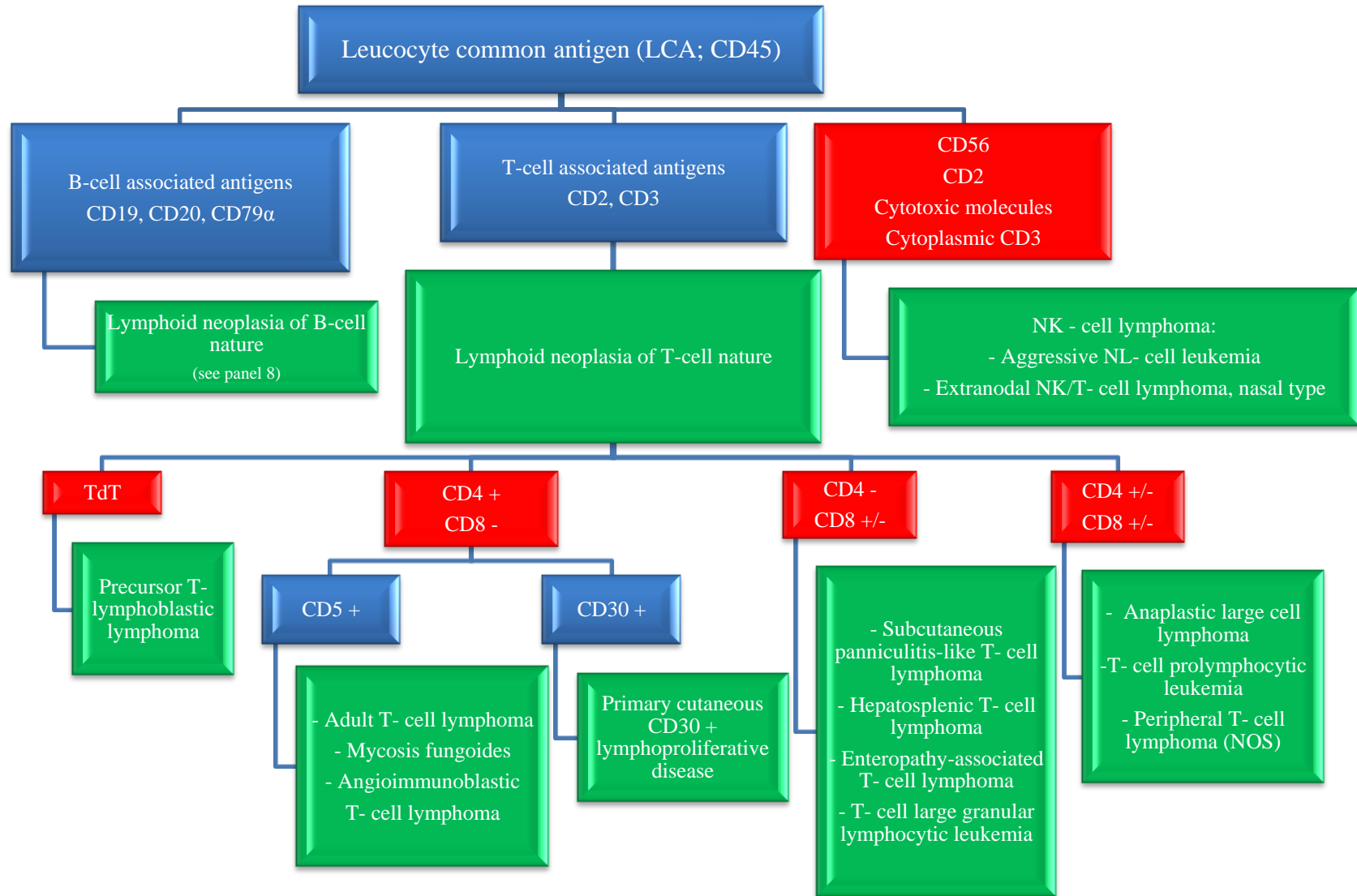
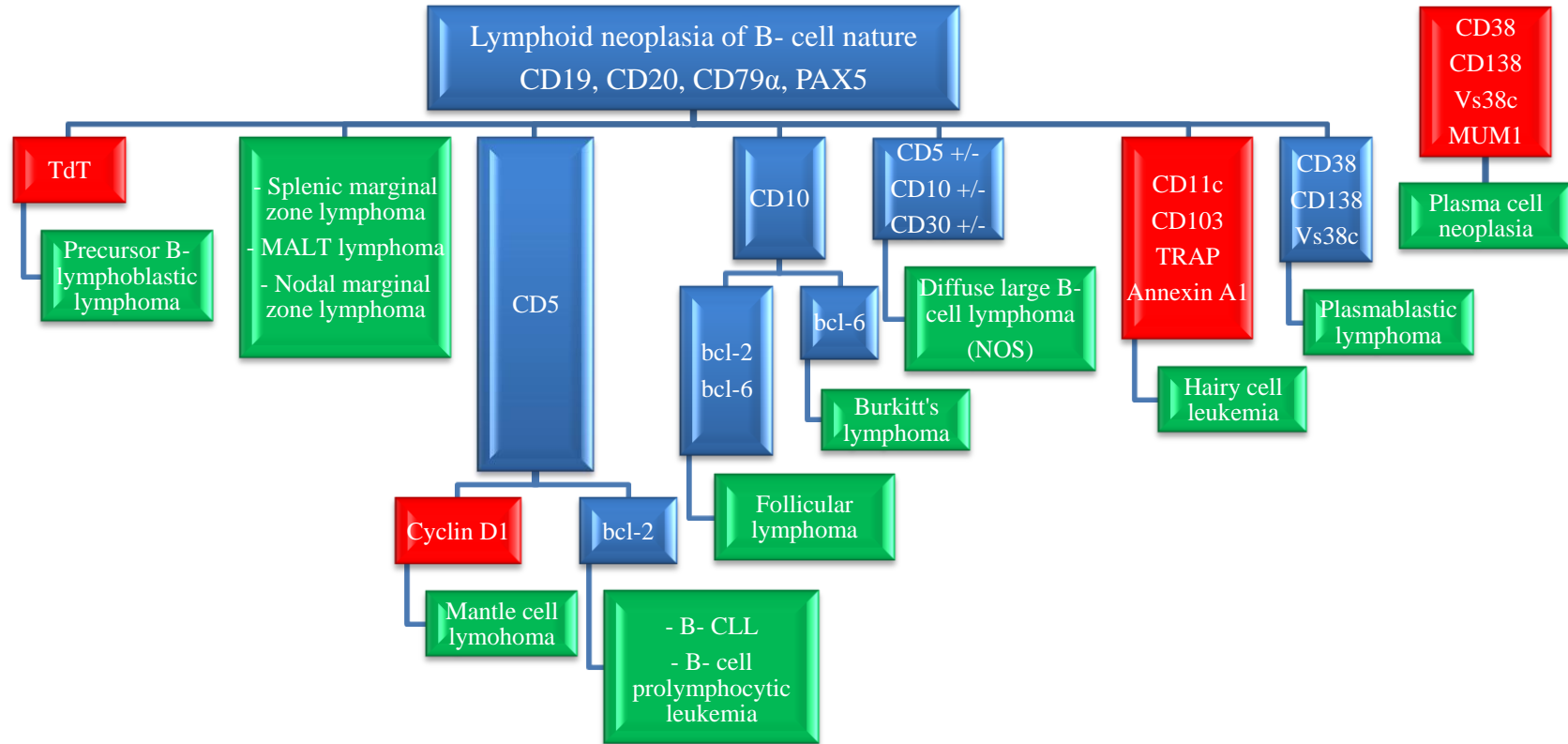
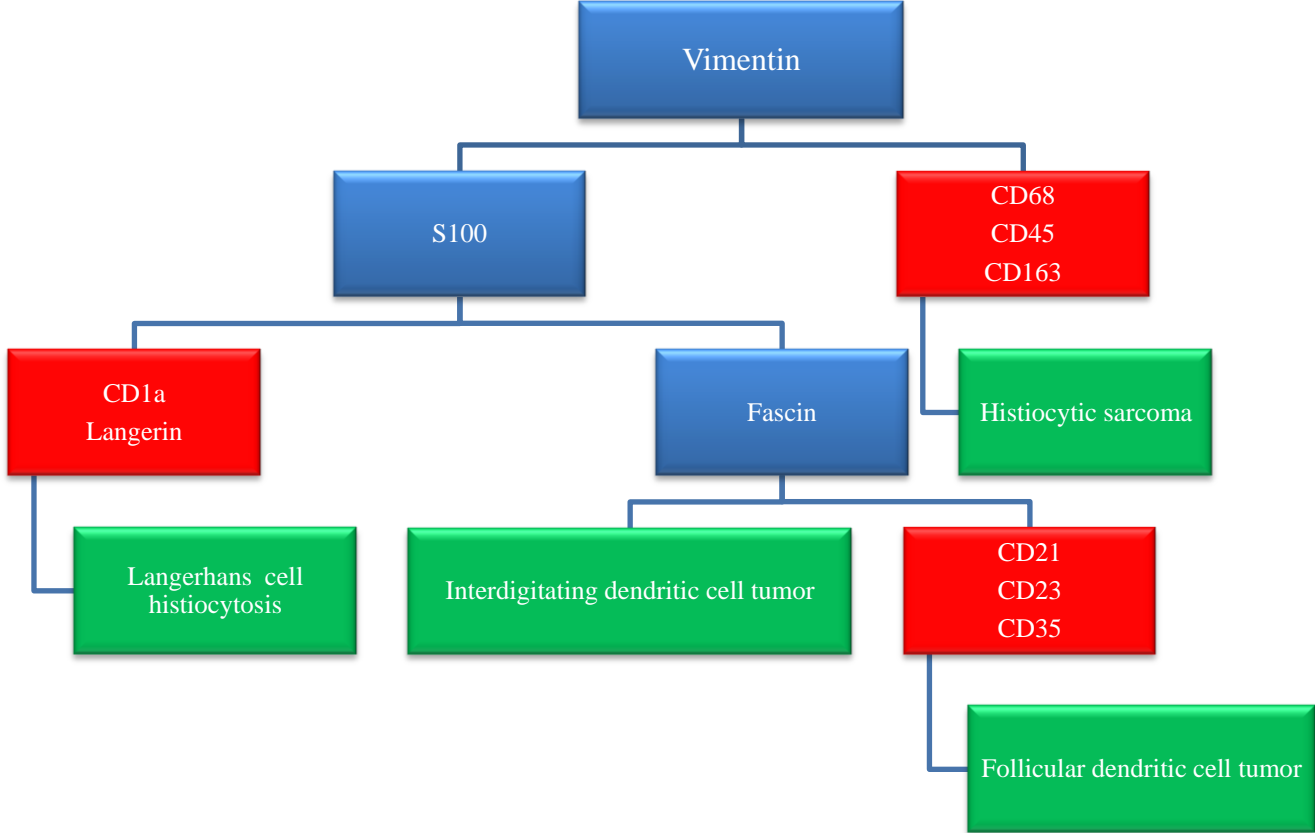


Figure 8: Algorithm for B-cell and plasma-cell neoplasia



Panel 9: Algorithm for histiocytic and dendritic cell tumors



5. Antibodies for immunohistochemical tumor diagnosis. Diagnostic approach targeting antigens with multilineal or atypical expression, benefits and pitfalls

A large number of monoclonal and polyclonal antibodies directed to different cellular and extracellular antigens covering a huge number of cell and tissue types at different stages of differentiation are used in modern immunohistochemistry. Many of the available antibodies are highly specific to cells or organs, a good example are CD3, CD20 Thyroglobulin and PSA but a large number of the available antibodies have a bi-expression or a wide expression spectrum. CD15, CD10, CD30, desmin and S100 are some of many antibodies with multilineage expression pattern. On the other hand, there are many tumors exhibiting a bilineage or atypical expression of different antigens. This phenomenon is described in various tissue and tumor types causing serious diagnostic pitfalls in the differential diagnosis between these tumors, especially tumors with ambiguous morphology such as spindle cell tumors and tumors with epithelioid differentiation. Known examples are synovial sarcoma with CD99, CD34 and cytokeratin expression, leiomyosarcoma with the aberrant expression of cytokeratins and epithelial membrane antigen as well as epithelioid sarcoma, metaplastic carcinoma and desmoplastic small round cell tumor.⁵

In this section, the most common antigens targeted in routine immunohistochemistry are listed according to their diagnostic use and expression range.

5.1. Antibodies for the diagnosis of epithelial neoplasia

Cytokeratins are the most important markers used for diagnosing epithelial neoplasia. Cytokeratins are intermediate filament proteins building an intracytoplasmic network between nucleus and cell membrane of epithelial cells. Cytokeratins are a complex family composed of more than 20 isotypes, divided into 2 types.^{6,7}

- Type I (acidic group) including cytokeratins 9-20.
- Type II (basic group) including cytokeratins 1-8.

Different cytokeratins are expressed in different epithelial types and at different stages of differentiation; consequently, different epithelial types have different specific cytokeratin expression

profiles, which usually remain constant after neoplastic transformation. Listed in this part are the most important cytokeratins used by pathologists in routine diagnosis. Epithelial membrane antigen (EMA) and carcinoembryonic antigen are not cytokeratins but mentioned in this section as they are also widely used in the diagnosis of epithelial neoplasia.

Pan-cytokeratin cocktails		
Expression pattern: Cytoplasmic		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Screening for epithelial neoplasia	Synovial sarcoma, epithelioid sarcoma, leiomyosarcoma, desmoplastic small round cell tumor, chordoma	Epithelial cells
Positive control: Appendix, tonsil		

Diagnostic approach: Pan-cytokeratin cocktails are very effective in screening for epithelial differentiation or epithelial neoplasia. Many cytokeratin-cocktails are successfully used for this purpose and the following cytokeratin-cocktails and clones are the most common ones used:

- AE1/AE3 cocktail, AE1 reacting with type I cytokeratins and AE3 with type II cytokeratins.
- Cytokeratin clone MNF116 reacts with cytokeratins 5/6/8/17 and 19.
- Cytokeratin clone CAM 5.2 reacts with cytokeratins 8/18/19.

Diagnostic pitfall: Cytokeratins are also expressed in various non-epithelial tissues and neoplasia such as mesothelial cells and mesothelioma, smooth muscle and smooth muscle tumors, germ cell tumors and some other tumors. The diagnosis of carcinoma based only on a positive pan-cytokeratin reaction is one of the sources of mistakes in tumor diagnosis. For appropriate diagnosis it is always advisable to determine the cytokeratin profile of the tumor and to search for other tissue specific markers. Ectopic benign epithelial structures in lymph nodes such as heterotopic ducts and glands in cervical, thoracic and abdominal lymph nodes in addition to Müllerian epithelial inclusions and endometriosis in pelvic lymph nodes must be kept in mind in screening lymph nodes for metastatic carcinoma or disseminated tumor cells.

Cytokeratin 5		
Expression pattern: Cytoplasmic		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Squamous cell carcinoma, mesothelioma, myoepithelial tumors	Myoepithelial cells in prostatic and breast carcinoma, basal like phenotype breast carcinoma, adrenocortical tumors.	Squamous epithelium, basal type epithelial cells, myoepithelial cells, transitional epithelium, mesothelial cells, cornea
Positive control: Tonsil		

Diagnostic approach: Cytokeratin 5, 6 and 14 are related cytokeratins expressed in stratified squamous epithelium and basal cells, myoepithelial and mesothelial cells. This expression spectrum makes those cytokeratins valuable markers for the diagnosis of squamous cell carcinoma. Cytokeratin 5 or a mixture of cytokeratins 5/6/14 is an important marker that clearly labels myoepithelial cells and myoepithelial tumors or tumors with myoepithelial component such as some salivary gland tumors. Highlighting the myoepithelial cells with this group of cytokeratins is essential for the interpretation of prostatic biopsies, as the myoepithelial cells are absent in neoplastic prostatic glands. An identical approach is also important to distinguish between simple hyperplasia, atypical ductal hyperplasia and ductal carcinoma in situ (DCIS) in breast specimens highlighting the myoepithelial and glandular cells with the cytokeratins 5/6/14 and 8/18, respectively. Cytokeratins 5/6/14 are highly expressed in mesothelial cells and are not suitable for discriminating between squamous cell carcinoma and mesothelioma in pleural or peritoneal biopsies or cytology. This group of cytokeratins is usually absent in gastrointestinal adenocarcinomas, germ cell tumors, prostatic carcinoma, thyroid tumors, hepatocellular and renal cell carcinomas. It is also noteworthy to mention the transcription factor p63 that highlights myoepithelial cells, basal and intermediate cells of squamous epithelium and urothelium with nuclear stain. P63 is now frequently used as an equivalent to the cytokeratins 5/6/14.¹⁰⁰

Cytokeratin 6		
Expression pattern: Cytoplasmic		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Squamous cell carcinoma	Poorly differentiated breast carcinoma (basal like phenotype breast carcinoma)	Suprabasal cells, hair, nail
Positive control: Tonsil		

Diagnostic approach: Cytokeratin 6 is usually used in routine immunohistochemistry as cocktail with cytokeratin 5.

Cytokeratin 7		
Expression pattern: Cytoplasmic		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Adenocarcinomas of lung, salivary glands, upper gastrointestinal tract, pancreas, biliary tract, breast, endometrium, transitional cell carcinoma, ovarian surface epithelial-stromal tumors.	Thyroid carcinoma, papillary and chromophobe renal cell carcinoma, mesothelioma, synovial sarcoma, Merkel cell carcinoma,	Epithelium of GIT, salivary glands, biliary tract, pancreas, lung, female genital tract, renal collecting ducts, transitional epithelium, mesothelial cells, thyroid follicle cells
Positive control: Appendix		

Diagnostic approach: Cytokeratin 7 is a type II cytokeratin expressed in the majority of ductal and glandular epithelium in addition to transitional epithelium of the urinary tract. Cytokeratin 7 is one of the main markers for the diagnosis of adenocarcinoma of different origin, hence it cannot be used alone to differentiate between primary and metastatic adenocarcinoma. An important diagnostic criterion is the co-expression of cytokeratin 7 with cytokeratin 20 (see diagnostic algorithms 5 & 6).⁹ Cytokeratin 7 is strongly expressed by mesothelial cells and not suitable for discriminating between adenocarcinoma and mesothelioma.

Diagnostic pitfall: In the differential diagnosis between adenocarcinoma and squamous cell carcinoma it is important to keep in mind that a minor component of cytokeratin7 positive cells can be found in squamous cell carcinoma of different locations such as head and neck, lung, esophagus and uterine cervix. Cytokeratin 7 can also be found in non-epithelial tumors such as the epithelioid component of synovial sarcoma. Cytokeratin 7 is usually absent in seminoma and yolk sac tumors, epidermal squamous cell carcinoma, prostatic carcinoma and pituitary tumors.

Cytokeratin 8		
Expression pattern: Cytoplasmic		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Adenocarcinoma of lung, GIT, pancreas, biliary tract, breast, endometrium, transitional cell carcinoma, hepatocellular carcinoma, renal cell carcinoma, prostatic carcinoma, neuroendocrine carcinoma	Leiomyosarcoma	Epithelium of GIT, salivary glands, biliary tract, pancreas, lung, female genital tract, hepatocytes, proximal renal tubules, transitional epithelium, mesothelial cells, smooth muscle cells, myofibroblasts, arachnoid cells
Positive control: Appendix		

Diagnostic approach: Both cytokeratins 8 and 18 are expressed in the early embryonal stages and found in simple epithelium. Cytokeratin 8 is usually positive in non-squamous carcinomas and accordingly cannot be used to discriminate between adenocarcinoma types.

Diagnostic Pitfall: Cytokeratin 8 is reported to react with several non-epithelial tissues and tumors such as smooth muscle cells and leiomyosarcoma.

Cytokeratin 10		
Expression pattern: Cytoplasmic		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Squamous cell carcinoma	Breast ductal carcinoma	Keratinizing epithelium
Positive control: Tonsil		

Diagnostic approach: Cytokeratin 10 is usually used as cocktail with cytokeratins 13 and / or 14 as marker for squamous cell carcinoma.

Cytokeratin 13		
Expression pattern: Cytoplasmic		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Squamous cell carcinoma		Mature non-keratinizing squamous epithelium, basal and intermediate cells of transitional epithelium
Positive control: Tonsil		

Diagnostic approach: Cytokeratin 13 is usually used in cocktails with cytokeratin 10 or cytokeratin 14 as marker for squamous cell carcinoma.

Cytokeratin 14		
Expression pattern: Cytoplasmic		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Squamous cell carcinoma, basal cell carcinoma, Hürthel cell tumors	Labeling of myoepithelial cells in prostatic carcinoma, basal like phenotype breast carcinoma	Keratinizing and non-keratinizing squamous epithelium, basal and myoepithelial cells in salivary glands, breast prostate and uterus, Hürthel thyroid cells
Positive control: Tonsil		

Diagnostic approach: Cytokeratin 14 is a helpful marker for the diagnosis of squamous cell carcinoma (see cytokeratin 5). In combination with cytokeratin 5 it is an excellent marker to stain the myoepithelial cells in breast and prostatic biopsies. The frequently used cytokeratin 34 β E12 to stain myoepithelial cells reacts with the cytokeratins 1, 5, 10 and 14.

Cytokeratin 18		
Expression pattern: Cytoplasmic		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Adenocarcinoma of lung, GIT, pancreas, biliary tract, breast, endometrium, transitional cell carcinoma, hepatocellular carcinoma, renal cell carcinoma, neuroendocrine carcinoma	Leiomyosarcoma, chordoma	Epithelium of GIT, salivary glands, biliary tract, pancreas, lung, female genital tract, hepatocytes, proximal renal tubules, transitional epithelium, mesothelial cells, smooth muscle cells, myofibroblasts, endothelial cells, arachnoid cells.
Positive control: Appendix		

Diagnostic approach: Cytokeratin 18 is expressed in simple epithelial cells and found in the majority of non-squamous carcinomas including adenocarcinoma of different origin, neuroendocrine carcinoma in addition to hepatocellular and renal cell carcinoma.

Diagnostic pitfall: Screening for intravascular tumor spread it is important to consider that cytokeratin 18 - which can be also a component of different cytokeratin cocktails - can be expressed by the endothelium of lymphatic and small venous vessels, which might mimic the intravascular tumor spread. Cytokeratin 18 is also found to be positive in smooth muscle tumors.

Cytokeratin 19		
Expression pattern: Cytoplasmic		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Adenocarcinoma of lung, GIT, pancreas, biliary tract, breast, endometrium, transitional cell carcinoma	Neuroendocrine tumors, papillary thyroid carcinoma, mesothelioma	Epithelium of GIT, salivary glands, biliary tract, pancreas, lung, female genital tract, transitional epithelium, mesothelial cells, thyroid follicle cells, basal squamous epithelium
Positive control: Appendix		

Diagnostic approach: Cytokeratin 19 is the smallest human cytokeratin found in both simple and complex epithelium. It is positive in the majority of carcinomas and has a limited use in differentiating between carcinoma types. Cytokeratin 19 strongly labels papillary thyroid carcinoma and can be used in differentiating between papillary and follicular thyroid carcinomas as follicular carcinoma is usually negative or very weak positive for cytokeratin 19.

Cytokeratin 20		
Expression pattern: Cytoplasmic		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Adenocarcinoma of GIT, pancreas and extrahepatic bile duct system, transitional cell carcinoma, mucinous ovarian tumors	Merkel cell carcinoma, mucinous pulmonary adenocarcinoma, hepatocellular carcinoma	Gastric- and colorectal epithelium, transitional epithelium
Positive control: Appendix		

Diagnostic approach: Cytokeratin 20 is a helpful marker in the differential diagnosis between different carcinoma types as the expression of this cytokeratin is restricted to a limited number of carcinomas. Cytokeratin 20 is constantly expressed by colorectal mucosa and colorectal adenocarcinomas, mucinous ovarian carcinoma as well as transitional cell carcinoma. Also characteristic is the dot like staining pattern in Merkel cell carcinoma. Cytokeratin 20 is constantly negative in squamous cell, breast, prostatic, and thyroid carcinoma and in endometrial adenocarcinoma and mesothelioma. The co-expression with cytokeratin 7 is an important diagnostic criterion for the differential diagnosis between different carcinoma types (see diagnostic algorithms 5 & 6).⁹

Epithelial membrane antigen (EMA; CD227, Episialin, MUC1)		
Expression pattern: Membranous / cytoplasmic		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Adenocarcinoma of different origin, anaplastic large cell lymphoma, lymphocyte predominant Hodgkin's lymphoma	Epithelioid sarcoma, meningioma, choroid plexus tumors, chordoma and parachordoma, plasmacytoma, mesothelioma,	Apical surface of glandular and ductal epithelial cells, activated T- cells, plasma cells, monocytes, follicular dendritic cells
Positive control: Appendix, tonsil		

Diagnostic approach: Epithelial membrane antigen (EMA) is a transmembrane glycoprotein with cytoplasmic and extracellular domains. EMA is highly expressed in different types of epithelial cells and neoplasms originating from these epithelial types. EMA is constantly negative in hepatocellular carcinoma, adrenocortical tumors and germ cell tumors i.e. seminoma, embryonal carcinoma and yolk sac tumor. EMA is also frequently expressed in the L&H cells of nodular lymphocyte predominant Hodgkin's lymphoma, making the EMA positivity a helpful criterion for the diagnosis since the L&H cells in this type of Hodgkin's lymphoma are CD30, CD15 and Fascin negative.

Diagnostic pitfall: EMA is not a specific epithelial marker, as it is widely expressed in other tumor and cell types such as anaplastic large cell lymphoma, plasma cell neoplasia, meningioma, mesothelioma, perineuroma, synovial and epithelioid sarcoma.

Carcinoembryonic antigen (CEA; CD66e)		
Expression pattern: Cytoplasmic / extracellular		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Gastrointestinal and pancreatic adenocarcinoma, pulmonary adenocarcinoma, cholangiocellular and hepatocellular carcinoma	Breast carcinoma, non-keratinizing lung squamous cell carcinoma, cervical adenocarcinoma, ovarian mucinous carcinoma, medullary thyroid carcinoma, adenocarcinoma of sweat glands, secretory meningioma	GIT mucosa, hepatocytes, thyroid C cells, granulocytes
Positive control: Colonic adenocarcinoma		

Diagnostic approach: Carcinoembryonic antigen (CEA) is a cell surface glycoprotein normally expressed by colonic mucosa of fetal colon and to a lesser degree in adult colonic mucosa. CEA is highly expressed in different carcinoma types of various origin. CEA negative tumors are of importance in the differential diagnosis. Prostatic carcinoma, endometrioid carcinoma, renal cell carcinoma, ovarian serous tumors, adrenal tumors, follicular and papillary thyroid carcinoma in addition to mesothelioma are constantly CEA negative. CEA is helpful in the differential diagnosis between mesothelioma and carcinoma, endocervical and endometrioid carcinoma, medullary and other types of thyroid carcinoma.

5.2. Antibodies for the diagnosis of pulmonary tumors

Diagnostic antibody panel for pulmonary carcinoma: Cytokeratin profile, TTF-1 and surfactant proteins.

Thyroid transcription factor-1 (TTF-1)		
Expression pattern: Nuclear		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Pulmonary carcinoma (adenocarcinoma, bronchioloalveolar carcinoma and small cell carcinoma), thyroid tumors	Non-pulmonary small cell carcinoma of different locations	Type II pneumocytes and Clara cells of lung, thyroid follicular and parafollicular C cells, diencephalon
Positive control: Thyroid tissue		

Diagnostic approach: Thyroid transcription factor (TTF-1) is a transcription factor that regulates the expression of different genes in the thyroid gland, lung and brain (diencephalon). TTF-1 is expressed in the majority of small cell carcinoma and adenocarcinoma of the lung, follicular, papillary and medullary thyroid carcinoma. A lesser degree of expression is found in large cell carcinoma of the lung and undifferentiated thyroid carcinoma but rarely in pulmonary squamous cell carcinoma.^{10, 11}

Diagnostic pitfall: The TTF-1 expression is rarely reported in different non-pulmonary small cell carcinomas such as urinary bladder small cell carcinoma and Merkel cell carcinoma.

Surfactant proteins		
Expression pattern: Cytoplasmic / membranous		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Pulmonary adenocarcinoma		Pneumocytes, bronchiolar cells
Positive control: Lung tissue		

Diagnostic approach: Surfactant proteins including surfactant proteins A, B, C and D in addition to surfactant precursors are lipoproteins synthesized by type II pneumocytes and bronchiolar cells. Antibodies to surfactant proteins are good markers for pulmonary adenocarcinoma. Pulmonary

squamous cell and large cell carcinomas and non-pulmonary adenocarcinomas and mesothelioma are usually negative for surfactants.

Diagnostic pitfall: The expression of some surfactants is described in some types of breast carcinoma. Macrophages in pleural effusion may be also positive to surfactant. The diagnosis of primary or metastatic pulmonary adenocarcinoma must be based on clinical data, microscopic appearance, cytokeratin profile and TTF-1 expression. The expression of surfactant and the lack of CDX-2- and steroid receptor expression are helpful to support the diagnosis of primary pulmonary carcinoma.

5.3. Antibodies for the diagnosis of gastrointestinal tumors

A. Diagnostic antibody panel for gastrointestinal carcinoma: Cytokeratin profile, CDX-1, CDX-2, CEA and Villin.

CDX-2		
Expression pattern: Nuclear		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Colorectal adenocarcinoma	Gastric adenocarcinoma, GIT carcinoids, islet pancreas tumors, ovarian mucinous adenocarcinoma, adenocarcinomas of urinary bladder	Intestinal epithelium, pancreatic epithelial cell
Positive control: Appendix		

Diagnostic approach: CDX-2 is a transcription factor protein regulating the differentiation and proliferation of intestinal epithelial cells. The expression of CDX-2 protein is found in gastrointestinal adenocarcinomas and gastrointestinal neuroendocrine tumors in different intensity whereas the highest frequency and intensity is characteristic for the colorectal adenocarcinomas.¹² The expression of CDX-2 is usually associated with the expression of cytokeratin 20. CDX-1 is analogous to CDX-2.

Diagnostic pitfall: the loss of CDX-2 expression can be noted in anaplastic high grade gastrointestinal adenocarcinomas. The expression of CDX-2 is reported in non-gastrointestinal adenocarcinomas, for instance a high expression level of CDX-2 is found in bladder adenocarcinoma derived from intestinal urachus, pancreatic adenocarcinoma, biliary adenocarcinoma and mucinous ovarian carcinoma. Pulmonary adenocarcinoma with mucinous differentiation can also be positive for CDX-2; this type of pulmonary adenocarcinoma is positive for cytokeratin 20 and lacks the expression of TTF-1.^{13,14} Some neuroendocrine tumors outside the GIT are also reported to be positive for CDX-2.¹⁰

B. Diagnostic antibody panel for gastrointestinal stromal tumors (GIST): CD34, CD117 (c-Kit), PDGFR- α and DOG-1

CD117 (c-kit; mast cell growth factor receptor; steel factor receptor)		
Expression pattern: Membranous		
Main diagnostic use	Expression in other tumors	Expression in normal cells
GIST, seminoma, mast cell disease,	Clear cell sarcoma, adenoid cystic carcinoma, small cell lung carcinoma, Ewing sarcoma, synovial sarcoma, osteosarcoma, angiosarcoma, AML, CML, renal oncocytoma, renal chromophobe carcinoma, thymic carcinoma, neuroblastoma, glioma	Interstitial cells of Cajal, hematopoietic progenitor cells, melanocytes, germ cells, glial and Purkinje cells, endothelial cells, mast cells, renal tubular cells, ovarian stroma and corpus luteum
Positive control: Brain tissue		

Diagnostic approach: CD117 (c-kit) has a very wide expression spectrum and is usually used as guide antibody for many tumors. The expression of CD117 is characteristic for more than 90% of GISTs and the co-expression with CD34 is an important diagnostic criterion for the diagnosis of gastrointestinal tumors (GIST). CD117 is also a very helpful marker for the diagnosis of seminoma and mast cell tumors.

Diagnostic pitfall: GISTs with epithelioid morphology or associated with PDGFR- α mutations are frequently negative for CD117 and antibodies to PDGFR- α and / or DOG-1 are commonly positive in CD117 negative GISTs.^{15, 86}

DOG-1		
Expression pattern: Membranous		
Main diagnostic use	Expression in other tumors	Expression in normal cells
GIST	Uterine leiomyoma, synovial sarcoma, esophageal squamous cell carcinoma, hepatocellular carcinoma	Cajal cells, gastric surface epithelium, salivary gland and pancreatic acini, gallbladder epithelium, myoepithelial cells.
Positive control: GIST		

Diagnostic approach: DOG-1 is a chloride channel protein introduced as a new marker for gastrointestinal stromal tumors. DOG-1 is highly specificity to GISTs and reacts with more than 90% this tumor identity. The expression spectrum of DOG-1 is different than that of CD117 but there is a high concordance between the expression of both markers in GISTs.^{16,17,18} Unlike CD117, DOG-1 is constantly negative in seminoma, myeloid and mast cell tumors.

Diagnostic pitfall: DOG-1 is found to be positive in up to 50% of intramural gastrointestinal leiomyomas but these are usually positive for actin and caldesmon.

CD34: CD34 is cell surface adhesion glycoprotein discussed in the section on vascular tumors. CD34 labels the majority of GISTs and is usually used in combination with CD117.

5.4. Antibodies for the diagnosis of exocrine pancreatic tumors

Diagnostic antibody panel for exocrine pancreatic tumors: Cytokeratin profile, CA19.9, CEA and DpC4.²⁹

CA19.9		
Expression pattern: Membranous / cytoplasmic		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Pancreatic and GIT carcinoma	Ovarian- and lung adenocarcinoma, renal cell and transitional cell carcinoma, mucoepidermoid carcinoma	Epithelium of breast ducts, lung, GIT, Hepatobiliary tract, salivary and sweet glands,
Positive control: Pancreatic tissue		

Diagnostic approach: CA19-9 stains strongly pancreatic and hepatobiliary adenocarcinomas but it is an unspecific marker with a very wide expression spectrum and may be expressed in carcinomas of different origin, however the diagnosis of primary pancreatic carcinoma must be supported by clinical and paraclinical data and histological appearance.

5.5. Antibodies for the diagnosis of liver tumors

A.Diagnostic antibody panel for hepatocellular tumors: Hep par 1, AFP, CD10, CD34 and cytokeratin profile.²¹

Hepatocyte specific antigen (Hep Par1)		
Expression pattern: Cytoplasmic		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Hepatocellular carcinoma, hepatoblastoma	Mucosal intestinal metaplasia, tumors with hepatoid differentiation	Hepatocytes
Positive control: Liver tissue		

Diagnostic approach: Hepatocyte specific antigen (Hep par 1) is a protein located on the mitochondrial membrane of hepatocytes, a specific marker for liver tissue and hepatocellular tumors.^{19, 20, 22} Hep par 1 is also a good marker to label lesions with intestinal metaplasia.

Diagnostic pitfall: Generally, extrahepatic tumors with hepatoid differentiation have the same immunoprofile as hepatocellular tumors and are positive for Hep par 1, AFP and CD10.⁶⁴ For the appropriate histopathologic diagnosis of primary or metastatic hepatocellular carcinoma - especially in small core biopsies - correlation with the other clinical and paraclinical data is required. False positive results in the immunostaining of liver tissue can be caused by the biotin activity of the hepatocytes, thus the inactivation of endogenous biotin is recommended. The use of a polymer detection system is also effective to eliminate the biotin background.

Alpha fetoprotein (AFP)		
Expression pattern: Cytoplasmic		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Hepatocellular carcinoma, Yolk sac tumor	Tumors with hepatoid differentiation, pancreatic-acinar cell carcinoma, pancreatoblastoma	Fetal liver
Positive control: Fetal liver		

Diagnostic approach: Alpha fetoprotein (AFP) is an oncofetal protein found in fetal liver, fetal gastrointestinal track, yolk sac and fetal plasma. AFP is also present in a very low concentration in

adult plasma. Hepatocellular carcinoma reveals, in the majority of cases, a high expression level of AFP and to a lesser degree in germ cell tumors, namely yolk sac tumor.

Diagnostic pitfall: It is important to consider that about 5% of hepatocellular carcinoma is negative for AFP. Low expression level of AFP is reported in pancreatic acinar cell carcinoma and pancreatoblastoma.

B. Diagnostic antibody panel for cholangiocellular carcinoma: Cytokeratin profile and CEA. These markers are discussed in other sections.

5.6. Antibodies for the diagnosis of breast tumors

Diagnostic antibody panel for breast carcinoma: Cytokeratin profile, estrogen- and progesterone receptors, mammaglobin, GCFPD-15, E-cadherin and HER-2.

Estrogen receptor		
Expression pattern: Nuclear		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Breast and endometrial carcinoma	Ovarian serous, mucinous and endometrioid carcinoma, transitional cell carcinoma, hepatocellular carcinoma	Breast and endometrial epithelium, endometrial stromal cells and myometrium
Positive control: Normal breast tissue		

Diagnostic approach: The expression of estrogen receptors (ER) is a good marker for the majority of breast carcinomas in addition to tumors of uterine- and ovarian origin. Good tissue fixation is required for optimal stain results. For all steroid receptors, any stain pattern other than nuclear must be interpreted as negative. The expression of ER is an important predictor for the response to the anti-hormone therapy.²

Diagnostic pitfall: The expression of ER depends on the tumor type and grade of tumor differentiation and is not restricted to the above mentioned organs but also can be found in other tumors such as hepatocellular carcinoma and transitional cell carcinoma. Additional markers such as mammaglobin, progesterone receptors, and GCDP15 as well as the cytokeratin profile are helpful to confirm the diagnosis.

Progesterone receptor		
Expression pattern: Nuclear		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Breast carcinoma endometrial carcinoma		Breast and endometrial cells, endometrium stromal cells
Positive control: Normal breast tissue		

Diagnostic approach: Progesterone receptors (PgR) are good marker for breast carcinomas and have more specificity than estrogen receptors as PgR are expressed in a limited number of tumors

such as endometrial carcinoma. Progesterone receptors are also an important predictor for the response to anti-hormone therapy.²

Diagnostic pitfall: similar to the estrogen receptors, the expression of PgR depends on the grade of tumor differentiation. High grade carcinomas are often negative for steroid receptors.

Mammaglobin		
Expression pattern: Cytoplasmic		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Breast carcinoma	Endometrioid adenocarcinoma, sweat gland carcinoma, salivary gland carcinoma	Adult breast
Positive control: Normal breast tissue		

Diagnostic approach: Mammaglobin is a low molecular protein, homologous to the human Clara cell protein expressed in adult breast tissue. Monoclonal antibodies to mammaglobin are good markers for tumors of breast origin but the expression of mammaglobin is found only in 80-90% of primary breast carcinoma and lymph node metastases.^{23, 24}

Diagnostic pitfall: similar to the other breast markers, the expression of mammaglobin is not restricted to breast tissue and breast tumors, but also found in few other types of adenocarcinoma.

Gross cystic disease fluid protein 15 (GCDFP-15)		
Expression pattern: Cytoplasmic		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Breast carcinoma	Salivary gland tumors, skin adnexal tumors, apocrine tumors, pulmonary adenocarcinoma	Apocrine-, lacrimal-, ceruminous- and Moll's- and cutaneous eccrine glands, serous cells of submandibular, sublingual and minor salivary glands, serous cells of nasal and bronchial glands
Positive control: Breast tissue / skin (apocrine cells)		

Diagnostic approach: Gross cystic disease fluid protein 15 (GCDFP-15) is a glycoprotein initially isolated from the human breast cystic fluid. 70-90% of primary and metastatic breast carcinomas are positive for GCDFP-15.

Diagnostic pitfall: GCDFP-15 is expressed in other apocrine-, eccrine- serous glandular epithelium and tumors derived from these glands.²⁵ Such tumors must be considered in the differential diagnosis.

HER-2 (c-erb-2)		
Expression pattern: Membranous		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Breast carcinoma, detection of HER-2 over-expression for immunotherapy	Ovarian and endometrial carcinomas	Breast epithelium
Positive control: HER-2 positive tumors / brain tissue		

Diagnostic approach: Human epidermal growth factor receptor-2 (HER-2) is a member of type 1 receptor tyrosine kinase family with domains on the cell surface, functioning as growth factor receptors. HER-2 gen amplification and over-expression detected by immunohistochemistry and FISH are important parameters for immunotherapy of breast carcinomas and other HER-2 positive carcinomas.²

Diagnostic pitfall: HER-2 is not a specific marker for breast cancer and found to be over- expressed only in 30-40% of breast carcinomas mainly in high-grade carcinoma; however HER-2 positive carcinomas are highly suspicious to be of breast origin.

5.7. Antibodies for the diagnosis of tumors of female genital tract

A. Diagnostic antibody panel for cervical, uterine and fallopian tube carcinoma: cytokeratin profile, CEA and steroid receptors.

B. Diagnostic antibody panel for uterine mesenchymal tumors: Smooth muscle markers, CD10 and steroid receptors.

C. Diagnostic antibody panel for ovarian tumors:

1. Diagnostic antibody panel for ovarian surface epithelial-stromal tumors: Cytokeratin profile, CEA, CA125 and steroid receptors.

CA125 (MUC16)		
Expression pattern: Membranous (luminal surface)		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Ovarian carcinoma (serous, endometrioid and clear cell carcinomas)	Lung-, breast-, GIT-, uterine- and seminal vesicle adenocarcinomas, yolk sac tumor, mesothelioma, anaplastic large cell lymphoma	Breast ductal epithelium, epithelium of lung, GIT, biliary tract, pancreas, female genital tract and apocrine glands, mesothelial cells
Positive control: Serous ovarian carcinoma		

Diagnostic approach: CA125 is a mucin-like glycoprotein expressed by glandular epithelium of different organs. CA125 is highly expressed in ovarian serous and clear cell carcinomas. Serum CA125 is also used to monitor the progression of ovarian carcinoma.

Diagnostic pitfall: CA125 is associated with different epithelial and non-epithelial malignancies and lacks the specificity to ovarian carcinoma.

2. Diagnostic antibody panel for ovarian germ cell tumors: CD117, PLAP, Oct-4, AFP, CD30, β hcG and cytokeratin profile (see testicular germ cell tumors).

3. Diagnostic antibody panel for ovarian sex cord-stromal tumors: Inhibin, anti-Müllerian hormone, melan A, CD99 (see testicular sex cord-stroma tumors).

5.8. Antibodies for the diagnosis of renal and urinary tract tumors

A. Diagnostic antibody panel for renal cell carcinoma: RCC, CD10, CD117, cytokeratin profile and vimentin.²⁶

Renal cell carcinoma marker (RCC; gp200)		
Expression pattern: Cytoplasmic		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Renal cell carcinoma (clear cell-, chromophobe- and papillary renal cell carcinoma)	Parathyroid adenoma, breast carcinoma, embryonal carcinoma	Renal proximal tubular brush border, epididymal tubular epithelium, breast parenchyma, thyroid follicles
Positive control: Renal tissue or Renal cell carcinoma		

Diagnostic approach: Renal cell carcinoma marker (RCC) is detected in about 90% of primary but less frequently in metastatic renal cell carcinoma, namely clear cell, chromophobe and papillary renal cell carcinoma, whereas the highest intensity is noted in the clear cell carcinoma.^{27, 28} Collecting duct carcinoma, sarcomatoid (spindle cell) carcinoma, oncocytoma, mesoblastic nephroma, nephroblastoma and transitional cell carcinoma are negative for RCC.

Diagnostic pitfall: RCC is occasionally detected in tumors other than renal cell carcinoma such as primary and metastatic breast carcinoma, embryonal carcinoma and parathyroid adenoma, which must be considered in the differential diagnosis.

CD10: CD10 is listed in detail in the lymphoma section, but it is also a helpful marker for the differential diagnosis of renal tumors. CD10 is positive in the majority of clear cell and papillary renal cell carcinomas in addition to collecting duct carcinoma but negative in chromophobe renal cell carcinoma.^{28,63}

Diagnostic pitfall: CD10 is also positive in tumors with similar morphology such as hepatocellular carcinoma and tumors of adrenal cortex.

B. Antibodies for the diagnosis of urinary tract tumors

Diagnostic antibody panel for transitional cell carcinoma: Cytokeratin profile (CK5/6/7/20), uroplakin and CD141 (thrombomodulin).

Uroplakins		
Expression pattern: Membranous		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Transitional cell tumors		Normal urothelium
Positive control: Urinary bladder mucosa		

Diagnostic approach: Uroplakins are transmembrane proteins expressed as rigid 0.2-0.5 μm plaques on the apical surface of mammalian urothelium and take part in the strengthening of the urothelial apical surface during distention of urinary bladder and urinary tract.^{30,31} Uroplakins are divided into 4 subtypes: Ia, Ib, II, and III, all of which are expressed by the urothelium of the urinary tract and the majority of tumors originate from the urothelium. Uroplakin subtypes Ia and II are specific for urothelium and are not detected in any tissue or carcinoma type other than transitional cell carcinoma. Both uroplakins are also negative in primary squamous cell carcinoma and adenocarcinoma of the urinary bladder.³² The uroplakin subtype Ib is detected in some other epithelial cells such as tracheal and bronchial epithelium and in the mucosa exhibiting squamous metaplasia. Uroplakin III is detected in prostatic glandular epithelium. Uroplakin and CD141 (thrombomodulin) are negative in renal cell carcinoma and can discriminate between transitional cell carcinoma and renal cell carcinoma.³³

Diagnostic pitfall: Antibodies to different uroplakins are specific markers for transitional cell carcinoma but these markers are generally positive in only about 60% of transitional cell carcinoma and the complete immunophenotyping including cytokeratin profile (CK5/6/7/20) and the expression of thrombomodulin and other tissue specific markers are required for appropriate diagnosis. The above mentioned tissue types positive for the uroplakins Ib and III and related tumors other than transitional cell carcinoma must be also considered in the differential diagnosis.

5.9. Antibodies for the diagnosis of male genital tract tumors

A. Diagnostic antibody panel for prostatic carcinoma: Cytokeratin profile, PSA, PAP, androgen receptors and PIN cocktail.

Prostate specific antigen (PSA)		
Expression pattern: Cytoplasmic		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Carcinoma of the prostate	Salivary duct carcinoma, small cell carcinoma	Prostatic secretory and ductal epithelium, periurethral glands, male anal glands
Positive control: Prostatic tissue		

Diagnostic approach: Prostate specific antigen (PSA) is one of the most specific markers for prostatic parenchyma and prostatic carcinoma. Metastatic carcinoma positive for pan-cytokeratin but negative for Cytokeratins 5/7 and 20 suggests suspected prostatic carcinoma and the expression of PSA will confirm the prostatic origin.

Diagnostic pitfall: About 10% of high grade prostatic carcinoma is PSA negative. In such cases other prostate specific markers such as prostate specific membrane antigen, prostatic acid phosphatase, and androgen receptors can be used to confirm the diagnosis. Low levels of PSA expression are reported in tumors other than prostatic carcinoma. We note weak expression of PSA in cases of salivary duct carcinoma. Weak expression of PSA is also reported in small cell carcinoma and breast carcinoma in addition to endometrioid carcinoma.

Prostatic acid phosphatase		
Expression pattern: Cytoplasmic		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Carcinoma of the prostate	Neuroendocrine tumors, intravascular large B- cell lymphoma	Prostatic secretory & ductal epithelium, periurethral glands, male anal glands
Positive control: Prostatic tissue		

Diagnostic approach: Prostatic acid phosphatase (PAP) is more sensitive but less specific than PSA for prostatic glands and prostatic carcinoma. PAP can be successfully used in a panel with PSA to classify metastases of unknown primary tumor.

Diagnostic pitfall: Similar to PSA, PAP can be also expressed in neuroendocrine carcinomas of different origin. This feature is important for the differentiating between poorly differentiated prostatic carcinoma, prostatic carcinoma with neuroendocrine differentiation and neuroendocrine tumors.

Androgen receptors		
Expression pattern: Nuclear		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Prostatic carcinoma	Osteosarcoma, apocrine breast carcinoma, Paget's disease	Prostate, apocrine and sebaceous glands, skin, oral mucosa
Positive control: Prostatic tissue		

Diagnostic approach: Androgen receptors (AR) are expressed in normal and neoplastic prostatic glands. Studies show that there is no direct correlation between the intensity of AR expression and the response to hormonal therapy.² The nuclear expression pattern of AR makes it useful for the immunohistochemical double stain with other antibodies with cytoplasmic or membranous expression pattern.

Diagnostic pitfall: The expression of AR is not restricted to prostatic carcinoma and can be found in other tumors such as breast carcinomas and apocrine carcinoma.

alpha-methylacyl-CoA racemase (AMARC, p504S)		
Expression pattern: Cytoplasmic		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Prostatic adenocarcinoma, high grade PIN	GIT adenocarcinoma, hepatocellular- and renal cell carcinoma, carcinoma of breast and ovaries, urothelial carcinoma, mesothelioma, lymphoma, pancreatic islet tumor, desmoplastic small round cell tumor	Periurethral glands, liver, salivary glands, renal tubular epithelium, pancreas epithelium, mesothelial cells
Positive control: Prostatic carcinoma		

Diagnostic approach: alpha-methylacyl-CoA racemase (also known as p504S) is an enzyme involved in the metabolism of branched-chain fatty acids expressed in various normal and neoplastic cells. p504S is over-expressed in prostatic carcinoma compared to benign prostatic glands.^{34,35} In combination with p63, alpha-methylacyl-CoA racemase (AMARC) is now widely used for the diagnosis of prostatic carcinoma (so called PIN cocktail). P63 is a myoepithelial marker exhibiting a nuclear stain.³⁶ This immunohistochemical double stain with the PIN cocktail can demonstrate 3 possible results:

- AMARC positive prostatic glands lacking the p63 positive myoepithelial cells; a combination characteristic of neoplastic glands.
- AMARC positive glands surrounded by p63 positive myoepithelial cells; characteristic of prostatic glands with high grade PIN.
- AMARC negative prostatic glands surrounded by p63 positive myoepithelial cells; characteristic of normal prostatic glands.

Diagnostic pitfall: The expression of AMARC is found in many neoplasia types and cannot be considered as a specific marker of prostatic tumors.³⁷

B. Antibodies for the diagnosis of testicular tumors

1. **Diagnostic antibody panel for germ cell tumors:** CD117, PLAP, Oct-3/4, AFP, CD30, β hcG and cytokeratin profile.

Placental alkaline phosphatase (PLAP)		
Expression pattern: Membranous		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Seminoma, embryonal carcinoma.	Proximal GIT tumors, lung and ovarian carcinoma. tumors with myogenic differentiation	Placental syncytiotrophoblasts, endocervical- and fallopian tube mucosa
Positive control: Seminoma		

Diagnostic approach: Placental alkaline phosphatase (PLAP) is expressed in several germ cell tumors such as seminoma, dysgerminoma, embryonal carcinoma, yolk sac tumor and gonadoblastoma. Since PLAP is not specific for any germ cell tumor type, a panel of antibodies is

required to differentiate between the PLAP positive germ cell tumors.^{39,40,43} The following profiles are helpful for the differential diagnosis of germ cell tumors:

- Characteristic profile for seminoma: CD117, PLAP and Oct-3/4.^{38,44}
- Characteristic profile for embryonal carcinoma: PLAP, AFP and CD30.
- Characteristic profile for yolk sac tumor: AFP, PLAP and pan-cytokeratin.
- Characteristic profile for choriocarcinoma: β hcG, PLAP and pan-cytokeratin.

Diagnostic pitfall: PLAP is rarely expressed in other carcinoma types such as breast and lung carcinoma. Additionally, it is important to consider that a cytoplasmic PLAP stain is reported in tumors with myogenic differentiation such as embryonal rhabdomyosarcoma and smooth muscle tumors.⁹¹

Oct-4		
Expression pattern: Nuclear		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Seminoma, intratubular germ cell neoplasia, embryonal carcinoma	Ovarian dysgerminoma, CNS germinoma	Germ cells (pluripotent germ cells)
Positive control: Seminoma		

Diagnostic approach: Oct-4 is a transcription factor involved in the differentiation of pluripotent germ cells. A high expression level of Oct-4 is characteristic for seminoma and embryonal carcinoma whereas spermatocytic seminoma lacks the Oct-4 expression.⁴¹ Oct4 labels the nuclei of the majority of the dysplastic cells of intratubular germ cell neoplasia but not the non-neoplastic testicular cells, making Oct4 a helpful and specific maker for intratubular germ cell neoplasia.⁴²

Human chorionic gonadotropin (HCG)		
Expression pattern: Cytoplasmic		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Syncytiotrophoblast in germ cell tumors (choriocarcinoma), non-seminomatous testicular tumors	Pulmonary large cell carcinoma and adenocarcinoma	Trophoblasts
Positive control: Placenta		

Diagnostic approach: Human chorionic gonadotropin is a hormone produced by syncytiotrophoblasts composed of α - and β -chains. The β -chain is more specific for syncytiotrophoblasts and related tumors as it is unique in structure. The α -chain shares amino acid sequences with other hormones such as LH, FSH and TSH of pituitary gland.

Diagnostic pitfall: The expression of HCG is reported in other non-syncytiotrophoblastic tumors such as pulmonary- and colonic carcinomas and also rarely lymphomas.⁴⁵

CD30: CD30 is listed in detail in the lymphoma section. Apart from hematologic malignancies, the expression of CD30 is characteristic for germ cell tumors, namely embryonal carcinoma and rarely yolk sac tumor.

2. Diagnostic antibody panel for sex cord-stromal tumors: Inhibin, anti Müllerian hormone, melan A, CD99.

Inhibin A		
Expression pattern: Cytoplasmic		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Sex cord-stromal tumors (granulosa cell tumor, Leydig-, Sertoli- and steroid cell tumor, thecoma and fibrothecoma), adrenocortical tumors	Choriocarcinoma and trophoblastic lesions	Sertoli cells, granulosa cells, theca interna, adrenal cortex, brain tissue
Positive control: Granulosa cell tumor / adrenal gland		

Diagnostic approach: Inhibin is a glycoprotein hormone composed of α and β subunits functioning as inhibitor for the follicle stimulating hormone (FSH) secretion. Antibodies to inhibin, Müllerian hormone and melan A are important diagnostic markers for sex cord-tumors.⁴⁶ Inhibin and Müllerian hormone are constantly negative in ovarian surface epithelial-stroma tumors, seminoma and embryonal carcinoma.

Diagnostic pitfall: Both inhibin and Melan A (MART-1) are also expressed in other tumors, mainly tumors of adrenal cortex. Furthermore melan A is widely used as melanoma marker.

5.10. Antibodies for the diagnosis of endocrine and neuroendocrine tumors

A. General endocrine and neuroendocrine markers: Chromogranin, synaptophysin, NSE, S100, PGP9.5, CD56.

The above mentioned antibodies are used to detect the neuroendocrine differentiation in tissue or tumors being examined, but none of these antibodies is a universal marker for the neuroendocrine differentiation; consequently, screening for such differentiation must include two or more antibodies. In our practice, we found that a mixture of chromogranin A and synaptophysin gives better results and superior stain intensity.

Chromogranin A		
Expression pattern: Cytoplasmic		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Neuroendocrine tumors: pituitary adenomas, medullary thyroid carcinoma, parathyroid adenoma / carcinoma, pheochromocytoma, islet cell tumors, Merkel cell carcinoma, small cell carcinoma, carcinoid and neuroendocrine carcinoma	Oligodendroglioma, neuroblastoma, PNET, paraganglioma	Neuroendocrine cells
Positive control: Appendix		

Diagnostic approach: Chromogranin and synaptophysin are the most commonly used neuroendocrine markers. Chromogranin A is one of the members of the chromogranin / secretogranin family that includes chromogranin A, chromogranin B (also known as secretogranin I) and chromogranin C (also known as secretogranin II). Chromogranins are expressed in almost all neuroendocrine cells and neuroendocrine tumors. The intensity of the immunostain depends on the number of neurosecretory granules in the cytoplasm of the cells examined; an example is small cell carcinoma, which synthesizes actively chromogranin, but because of paucity of cytoplasm and scarcity of neurosecretory granules, shows usually very weak chromogranin stain.

Synaptophysin		
Expression pattern: Synaptophysin		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Neuroendocrine tumors: pituitary adenomas, medullary thyroid carcinoma, parathyroid adenoma / carcinoma, pheochromocytoma, islet cell tumors, small cell carcinoma, carcinoid and neuroendocrine carcinoma	Medulloblastoma, retinoblastoma, neurocytoma, ependymoma, neuroblastoma, adrenocortical tumors, Merkel cell carcinoma	Neuronal and neuroendocrine cells, carotid body cells, adrenal cortex and medulla
Positive control: Appendix		

Diagnostic approach: Synaptophysin is a transmembrane calcium-binding glycoprotein present in the presynaptic vesicles. Synaptophysin is a wide spectrum marker for neuroendocrine cells and tumors with neuroendocrine differentiation. The double stain with an antibody mixture of chromogranin and synaptophysin will increase the sensitivity and improve the stain results.

Neuron specific enolase (NSE) γ- subunit		
Expression pattern: Cytoplasmic		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Neuroectodermal and neuroendocrine tumors	Melanoma, Merkel cell carcinoma, meningioma, renal cell carcinoma	Neurons, neuroendocrine cells, megakaryocytes, T-lymphocytes, smooth and striated muscle
Positive control: Appendix		

Diagnostic approach: Neuron specific enolase (NSE) is a glycolytic enzyme catalyzing the reaction pathway between 2-phospho-glycerate and phosphophenol pyruvate. Enolases are homo- or heterodimers composed of the three subunits: alpha (α) subunit, beta (β) subunit and gamma (γ) subunit, whereas antibodies to the γ -subunit are the most commonly used. The γ -subunits are primarily expressed in neurons, in normal and in neoplastic neuroendocrine cells, but they can be also expressed in megakaryocytes, T- lymphocytes in addition to striated and smooth muscle cells.

Diagnostic pitfall: NSE has a low specificity to neuroendocrine tumors, usually used as a screening marker and the diagnosis must be supported by other more specific markers.

S100		
Expression pattern: Cytoplasmic / nuclear		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Melanomas, schwannoma, histiocytic (Langerhans cell) neoplasia, neuroendocrine tumors	Liposarcoma, malignant peripheral nerve sheath tumors, chondrosarcoma and chondroblastoma, clear cell sarcomas, myoepithelial tumors, granulosa cell tumor	Cells of neural crest (glial cells, Schwann cells, melanocytes and nevus cells), chondrocytes, adipocytes, myoepithelial cells, macrophages, adrenal medulla and paraganglia, Langerhans cells, dendritic cells
Positive control: Appendix		

Diagnostic approach: S100 belongs to the family of calcium binding proteins expressed in different cells of different histogenesis and tumors originating from these cells. S100 is a widely used broad spectrum marker and different polyclonal or monoclonal antibodies directed to various subunits of the S100 protein are available for this purpose.

Diagnostic pitfall: It is important to consider that S100 is a screening marker that lacks the specificity and the final diagnosis must be confirmed by additional more specific markers.

Further endocrine- and neuroendocrine markers such as CD56 and PGP9.5 are listed in detail in other sections.

B. Diagnostic antibody panel for neuroendocrine and small cell carcinomas: Cytokeratin profile, chromogranin, synaptophysin, NSE, S100 and CD56.^{7,47,48}

C. Diagnostic antibody panel for thyroid neoplasia: TTF-1, thyroglobulin, calcitonin, cytokeratin profile.⁴⁹

Thyroid transcription factor (TTF-1): Thyroid transcription factor (TTF-1) is mentioned in detail among the markers for pulmonary carcinomas. In addition to pulmonary carcinomas, the expression of TTF-1 is characteristic for thyroid carcinomas and strongly expressed in follicular-, papillary and medullary thyroid carcinomas but rarely in the undifferentiated (anaplastic) thyroid carcinoma.

Thyroglobulin		
Expression pattern: Cytoplasmic		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Follicular and papillary thyroid carcinomas		Thyroid follicular cells
Positive control: Thyroid tissue		

Diagnostic approach: Thyroglobulin is a glycoprotein synthesized by the thyroid follicular cells. Thyroglobulin is a specific diagnostic marker for thyroid follicular cells and thyroid follicular cell neoplasia. It is recommended to use thyroglobulin in a panel with TTF-1 to discriminate between pulmonary and thyroid carcinoma. Anaplastic thyroid carcinoma is usually negative for thyroglobulin. Thyroid parafollicular C cells and related neoplasia constantly lack the expression of thyroglobulin.

Calcitonin		
Expression pattern: Cytoplasmic		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Medullary thyroid carcinoma	Neuroendocrine carcinoma	Thyroid parafollicular (C) cells
Positive control: Thyroid tissue / medullary thyroid carcinoma		

Diagnostic approach: Calcitonin is a polypeptide hormone synthesized by the parafollicular (C) thyroid cells. Calcitonin is a specific marker for the parafollicular cells and tumors originating from these cells, namely medullary thyroid carcinoma. Tumors originating from the thyroid follicular cells are negative for calcitonin but also positive for TTF-1. Best stain results are obtained using monoclonal antibodies.

Diagnostic pitfall: Neuroendocrine tumors such as pheochromocytoma are rarely reported to be positive for calcitonin but these tumors are usually negative for TTF-1.

D. Diagnostic antibody panel specific for pancreatic endocrine tumors: Insulin, gastrin, glucagon, somatostatin, vasoactive intestinal polypeptide (VIP) and human pancreatic polypeptide (hPP).

E. Antibodies for the diagnosis of adrenal gland tumors

1. Diagnostic antibody panel for adrenocortical adenoma / carcinoma: adrenal binding protein, DAX-1, inhibin, melan A, calretinin, synaptophysin, D2-40 and WT-1.

Adrenal 4 binding protein (Ad4BP, SF-1)		
Expression pattern: Nuclear		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Adrenocortical tumors	Sex cord-stromal tumors	Adrenal cortex, stromal cells of the spleen, gonadotrophic cell in the anterior pituitary gland, ovarian stromal cells
Positive control: Adrenal gland		

Diagnostic approach: Adrenal 4 binding protein (Ad4BP), also known as steroid factor 1 (SF-1), is a transcription factor involved in the steroidogenesis expressed in the adrenal cortex and related tumors. Ad4BP is constantly negative in renal cell and hepatocellular carcinomas, melanoma and pheochromocytoma. A very little amount of this antibody was available for us, so we have limited experience concerning its diagnostic value. Generally the positivity to synaptophysin, melan A, inhibin, D2-40 and calretinin and the co-expression of vimentin and cytokeratin 5 will support the adrenocortical origin of the tumor.^{7,50,57}

Diagnostic pitfall: Clinical and paraclinical data must be considered for the diagnosis of metastatic adrenocortical carcinoma as the immunoprofile of sex cord-stromal tumors is very similar to that of adrenocortical tumors.

2. Antibodies for the diagnosis of tumors of adrenal medulla and extra-adrenal paraganglia

- **Diagnostic antibody panel for pheochromocytoma and extra-adrenal paraganglia:** Chromogranin, Synaptophysin, CD56, NSE, S100. These antibodies are listed in detail in other sections.
- **Diagnostic antibody panel for neuroblastoma:** NB84, synaptophysin, CD56, PGP9.5 and neurofilaments.⁵²

NB84		
Expression pattern: Membranous		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Neuroblastoma	Ewing's sarcoma / PNET, medulloblastoma, desmoplastic small round cell tumor	
Positive control: Neuroblastoma		

Diagnostic approach: NB84 is a membranous antigen isolated from the human neuroblastoma cells. It stains about 100% of differentiated and about 90% of undifferentiated neuroblastomas. NB894 is more sensitive but less specific than synaptophysin.⁵¹ For an appropriate diagnosis of adrenal or extra adrenal tumors, a panel of 3-4 of the above motioned antibodies is recommended.

Diagnostic pitfall: NB84 is found to be positive in other tumors with similar morphology including PNET and desmoplastic small round cell tumor. To exclude these tumors, an antibody panel including CD99 and cytokeratins is required. It is important to consider that about 5% of undifferentiated neuroblastoma lacks the expression of NB84.

5.11. Antibodies for the diagnosis of mesothelioma

Diagnostic antibody panel for mesothelioma: Calretinin, thrombomodulin (CD141), mesothelin, podoplanin, WT-1 and cytokeratin profile.^{53,54,55}

Calretinin		
Expression pattern: Cytoplasmic		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Mesothelioma, mast cell lesions	Squamous cell carcinoma, transitional cell carcinoma, granular cell tumor, adrenocortical tumors, ovarian sex cord-stromal tumors, fibrosarcoma	Central and peripheral neural tissue, mesothelial cells, mast cells, Leydig and Sertoli cells, adrenal cortex, endometrium, eccrine glands, thymus
Positive control: Appendix		

Diagnostic approach: Calretinin is an intracellular calcium binding protein expressed in various epithelial, mesenchymal and neurogenic tissue types. Calretinin is strongly expressed in normal and neoplastic mesothelial cells and considered to be one of the important markers for mesothelioma.

Diagnostic pitfall: Calretinin has a wide expression spectrum and the calretinin positivity alone cannot justify the diagnosis of mesothelioma.

CD141 (Thrombomodulin)		
Expression pattern: Membranous		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Mesothelioma, transitional cell carcinoma	Squamous cell carcinoma, trophoblastic tumors, vascular tumors, synovial sarcoma	Endothelial cells, urothelium, mesothelial cells, keratinizing epithelial cells, meningeal cells, platelets / megakaryocytes, monocytes, neutrophils, smooth muscle cells, syncytiotrophoblasts, synovial lining cells
Positive control: Appendix		

Diagnostic approach: CD141 (thrombomodulin) is a transmembrane glycoprotein expressed in different cell types and may be used as a screening antibody for mesothelioma, transitional cell- and squamous cell carcinoma and vascular tumors. CD141 cannot discriminate between these tumors and it is usually negative in sarcomatoid mesothelioma. CD141 also important because it is constantly negative in renal cell and prostatic carcinoma, gastrointestinal adenocarcinoma and endometrioid carcinoma.

Mesothelin		
Expression pattern: Membranous		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Mesothelioma, ovarian surface carcinomas	Adenocarcinoma, acinar cell carcinoma and squamous cell carcinoma	Mesothelial cells, renal tubules, tracheal and tonsil mucosa
Positive control: Appendix		

Diagnostic approach: Mesothelin is a glycoprotein localized on the cell surface of mesothelial and many epithelial cells. It is also expressed in mesothelioma and many carcinoma types including ovarian carcinoma and pancreatic adenocarcinomas. Generally, mesothelin is a screening antibody and cannot be considered a specific mesothelioma marker.

Wilms tumor protein-1 (WT-1)		
Expression pattern: Nuclear		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Nephroblastoma, mesothelioma, malignant melanoma, metanephric adenoma, ovarian serous carcinoma	Acute myeloid leukemia, desmoplastic small round cell tumor, endometrial stromal sarcoma, uterine leiomyosarcoma, Sex cord-stromal tumors (granulosa cell tumor, thecoma), rhabdoid tumor, breast carcinoma	Renal tissue, mesothelial cells, granulosa cells, Sertoli cells, fallopian tube, endometrial stroma, spleen, breast tissue
Positive control: Appendix		

Diagnostic approach: Wilms tumor protein-1 (WT1) is a transcriptional regulator involved in development of tissues from the inner layer of intermediate mesoderm. WT-1 is expressed in many types of neoplasia. WT-1 is one of the important mesothelioma markers and because of its nuclear expression pattern can be used as the double stain in combination with other markers exhibiting membranous stain. WT-1 is a helpful marker in the differentiating between WT-1 positive tumors and many WT-1 negative tumors including rhabdomyosarcoma, neuroblastoma, PNET tumor group, endometrial carcinoma, ovarian mucinous and clear cell carcinoma.

Podoplanin: Podoplanin (also known as D2-40) is a mucoprotein expressed on the membrane of lymphatic endothelium discussed in the section on vascular tumors. Podoplanin is not a specific marker for lymphatic vessels and is also expressed in mesothelial cells and mesothelioma in addition to many other tumors such as germ cell tumors and different mesenchymal tumors.^{56,57}

Diagnostic criteria for mesothelioma:

Finally it is important to point out that mesothelioma has no constant morphologic appearance and may demonstrate epithelioid, sarcomatoid, desmoplastic or mixed (biphasic) differentiation pattern, demonstrating different immunophenotypes. It is always advisable to exclude other tumors using more specific markers such as TTF-1, CDX-2, CEA, steroid receptors and CD15, which are constantly negative in mesothelioma. Generally, it is advisable to confirm the diagnosis of mesothelioma by 3-4 mesothelioma markers.⁵³

5.12. Antibodies for the diagnosis of lymphoma

A. Screening antibodies for lymphoma: CD45 (LCA), TdT, B- cell markers and T- cell markers.^{58,59,60}

CD45 (LCA)		
Expression pattern: Membranous		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Lymphoma / Leukemia	Granulocytic sarcoma, histiocytic sarcoma, dendrocytoma, interdigitating dendritic cell sarcoma, giant cell tumor of tendon sheet,	Hematopoietic cells including B- and T- lymphocytes, monocytes, macrophages and mast cells; dendritic cells, medullary thymocytes, fibrocytes
Positive control: Appendix		

Diagnostic approach: CD45, also known as leukocyte-common antigen (LCA), is a family of high molecular mass integral membrane glycoprotein molecules expressed on all hematopoietic cells except mature red cells and their immediate progenitors, megakaryocytes and platelets.

Diagnostic pitfall: less than 3% of B- cell lymphoma, about 10% of T- cell lymphoma and about 30% of precursor B- and T- lymphoblastic lymphomas (ALL) lack the expression of CD45. In suspicious cases the use of other lymphoid markers is required. Membranous CD45 stain is reported in very rare cases of undifferentiated, neuroendocrine and small cell carcinomas. Necrotic carcinomas can also imitate a membranous LCA positivity.

TdT (Terminal deoxynucleotidyl transferase)		
Expression pattern: Nuclear		
Main diagnostic use	Expression in other tumors	Expression in normal cells
B- and T- ALL	AML, CML, Merkel cell carcinoma	B- and T- cell precursors, cortical thymocytes
Positive control: ALL		

Diagnostic approach: Terminal deoxynucleotidyl transferase (TdT) is a DNA nuclear polymerase, catalyzing the template independent polymerization of deoxynucleotidyl triphosphates to double-

stranded gene segment DNA. TdT is mainly expressed in primitive B- and T- lymphocytes. Antibodies to TdT are specific markers for precursor cell lymphomas of T- and B- cell origin, namely acute lymphoblastic leukemia and lymphoma.

Diagnostic pitfall: It is always important to remember that TdT may be positive in some types of acute myeloid leukemia (AML), blast crisis of chronic myeloid leukemia (CML) and Merkel cell carcinoma.^{61,62}

CD 5 and CD10 are further helpful lymphoma markers. As they do not have lineage specificity, we mention them in the section on general markers.

CD10 (CALLA)		
Expression pattern: Membranous		
Main diagnostic use	expression in other tumors	Expression in normal cells
Burkitt's lymphoma, acute lymphoblastic lymphoma/leukemia, angioimmunoblastic lymphoma, endometrial stromal tumors, renal cell carcinoma	Follicular lymphoma, B- cell lymphoma, plasma cell neoplasia, hepatocellular carcinoma, transitional cell carcinoma, prostatic carcinoma, melanoma, placental site trophoblastic tumor, mesothelioma, rhabdomyosarcoma, leiomyosarcoma, Ewing's sarcoma, solitary fibrous tumor	Pre-B- and pre-T- cells, cells of germinal centers, granulocytes, adrenal cortex, endometrial stroma cells, hepatocytes and bile duct canaliculi, cells of proximal renal tubules and glomerular epithelial cells , endothelial cells, myoepithelial cells, fibroblasts, brain tissue, choroid plexus, fetal intestinal epithelium
Positive control: Appendix / tonsil		

Diagnostic approach: CD10 is a zinc-dependent cell membrane metalloprotein involved in the postsecretory processing of neuropeptides and vasoactive peptides. Despite the name of CD10 as the common acute lymphoblastic leukemia antigen (CALLA) it is not a cell line- or tumor specific marker and it is expressed in a long list of tissue and tumor types of lymphoid, epithelial and mesenchymal origin mentioned in the above table.^{63,64} In diagnostic immunohistochemistry, CD10 must be used in a panel with other tissue and cell specific markers.⁸⁴

CD5		
Expression pattern: Membranous		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Mantle cell lymphoma	B- CLL, T- ALL, T- cell lymphoma, prolymphocytic leukemia, atypical thymoma and thymic carcinoma	T- cells, subset of B- cells of mantle zone of spleen and lymph nodes
Positive control: Appendix / tonsil		

Diagnostic approach: CD5 is a glycoprotein receptor expressed in the majority of T- lymphocytes and different T- cell neoplasia such as T- ALL, adult and peripheral T- cell lymphoma, Mycosis fungoides and T- cell large granular lymphocytic leukemia. The expression of CD5 is not restricted to T- lymphocytes but it is also found in a small subset of B- lymphocytes and lymphomas of B- cell origin mainly mantle cell lymphoma and B- CLL.

Diagnostic pitfall: The expression of CD5 is not limited to lymphoid tissue as the expression of CD5 is a diagnostic marker for atypical thymoma and thymic carcinoma, a focal weak expression of CD5 can be also found in mesothelioma, transitional-, squamous cell carcinoma and adenocarcinomas of different origin.⁷⁰

B. Diagnostic antibodies for B- cell neoplasia: CD19, CD20, CD79a, PAX-5, cyclin D1 and TdT.^{59,60,84}

CD19 (B4)		
Expression pattern: Membranous		
Main diagnostic use	expression in other tumors	Expression in normal cells
B- cell lymphoma / leukemia	AML (M0), blast phase of CML	B- cells, follicular dendritic cells
Positive control: Appendix / tonsil		

Diagnostic approach: CD19 is an early naïve B- lymphocyte antigen, which remains through the B- lymphocyte differentiation stages and disappears in the plasma cell stage. CD19 is an excellent B- lymphocyte marker and antibodies to CD19 are available for both flow cytometry and paraffin histology.⁶⁵

CD20 (B1 antigen)		
Expression pattern: Membranous		
Main diagnostic use	expression in other tumors	Expression in normal cells
B- cell lymphoma / leukemia		B- cells, follicular dendritic cells
Positive control: Appendix / tonsil		

Diagnostic approach: CD20 is a transmembrane protein acting as receptor during B- cell activation and differentiation. CD20 is expressed in B- cells after CD19 in the naïve B- lymphocytes and remains until late stages of B- lymphocyte differentiation but disappears in the plasma cell stage.

Diagnostic pitfall: CD20 is a pan B- lymphocyte marker but some types of B- cell lymphomas are negative to CD20 or show a very weak expression level; consequently in doubtful cases, it is important to use 2 B- cell markers to assure or exclude the B- cell origin of the neoplasia, good combinations are CD20/CD19 and CD20/PAX-5. Generally, the expression of CD20 is restricted to B- lymphocytes but rare cases of CD20 expression in peripheral T- cell lymphoma are reported. Another diagnostic pitfall is the interpretation of CD20 stain in patients after the specific CD20 immunotherapy (rituximab).

CD79a		
Expression pattern: Membranous		
Main diagnostic use	Expression in other tumors	Expression in normal cells
B- cell leukemia / lymphomas	Acute promyelocytic leukemia (FAB-M3), multiple myeloma	B- cells, small population of CD3+ T- cells, subset of endothelial cells
Positive control: Appendix / tonsil		

Diagnostic approach: CD79a is a disulphide linked heterodimer associated with the membrane-bound immunoglobulin; it appears in the pre B- lymphocyte stage and persists until the plasma cell development, whereas the majority of normal and neoplastic plasma cells are positive for CD79a. CD79a exhibits a membranous stain but plasma cells may also show a cytoplasmic stain pattern. The expression of CD79a is independent of the expression of CD20 and remains positive after the anti-CD20 immunotherapy.

Diagnostic pitfall: CD79a is less reliable than CD20 for the diagnosis of B- cell lymphoma, as it is positive in a small fraction of T- ALL, AML (FAB-M3) and the majority of plasma cell neoplasia.

PAX-5 (B- cell-specific activator protein, BSAP)		
Expression pattern: Nuclear		
Main diagnostic use	Expression in other tumors	Expression in normal cells
B- cell lymphoma / leukemia, Reed-Sternberg cells in classic Hodgkin's lymphoma	Merkel cell carcinoma, alveolar rhabdomyosarcoma, small cell carcinoma, Wilms tumor, glioblastoma and neuroblastoma	Pre B- to mature B- cells
Positive control: Appendix / tonsil		

Diagnostic approach: PAX-5 is a B- cell specific transcription factor expressed in the early pro-B, pre-B and naïve stages of B- cell development until the mature B- cells.⁶⁶ PAX-5 is also expressed in the L&H cells of nodular lymphocyte predominance Hodgkin's lymphoma. T- lymphocytes, plasma cells and macrophages are constantly PAX-5 negative.

Diagnostic pitfall: PAX-5 can be positive in some tumors resembling lymphoma such as Merkel cell carcinoma, small cell carcinoma and also rarely in acute lymphoblastic lymphoma of T- cell origin.⁶⁷ PAX-5 maybe also expressed in acute myeloid leukemia, mainly the type associated with the t(8;21)(q22;q22) translocation. PAX-5 positivity is reported in rare cases of breast, endometrial and transitional carcinomas in addition to alveolar rhabdomyosarcoma.^{68,69}

Cyclin D1 (bcl-1)		
Expression pattern: Nuclear		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Mantle cell lymphoma	Inflammatory pseudotumor (myofibroblastic tumor), parathyroid adenoma/carcinoma, hairy cell leukemia, pulmonary adenocarcinoma, breast carcinoma	Cells in the G ₁ phase of cell cycle
Positive control: Mantle cell lymphoma		

Diagnostic approach: Cyclin D1 (also known as bcl-1) is a cell cycle regulatory protein involved in the in the regulating of cyclin-dependent kinases in the G₁ of the cell cycle. Cyclin D1 is not a lymphoid marker and found to be expressed in a number of non-lymphoid tumor types. As a result

of the t(11;14) translocation associated with mantle cell lymphoma that causes the over-expression of cyclin D1, cyclin D1 it is considered to be a characteristic marker for this lymphoma type, usually in combination with CD5 and other B- cell markers.^{2,71,84} A subset of t(11;14) associated multiple myeloma is also found to be positive for cyclin D1.

bcl-6		
Expression pattern: Nuclear		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Follicular lymphoma, anaplastic CD30+ large cell lymphoma	Follicular lymphoma (in interfollicular area), Burkitt's lymphoma, L&H cells in nodular lymphocyte predominance Hodgkin's lymphoma	Germinal centers of lymph nodes
Positive control: Appendix / tonsil		

Diagnostic approach: bcl-6 is a transcriptional regulator expressed in germinal center cells and in tumors of germinal center origin such as follicular lymphoma and Burkitt's lymphoma.⁸⁴ Bcl-6 is usually negative in T-cell lymphoma.

bcl-2		
Expression pattern: Cytoplasmic / membranous		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Follicular lymphoma	Majority of B- cell lymphomas, subset of T- cell lymphoma, solitary fibrous tumor, synovial sarcoma, hemangiosarcoma, neurofibroma, schwannoma, nasopharyngeal carcinoma, dermatofibrosarcoma protuberans, spindle cell lipoma, rhabdomyosarcoma	Small B- lymphocytes in mantle zone and cells within T- cell areas, medullary cells in thymus
Positive control: Appendix / tonsil		

Diagnostic approach: The bcl-2 proteins are encoded by the bcl-2 gene on chromosome 18q21, which consists of 3 exons. The bcl-2 gene is transcribed into 3 mRNA variants, which are translated into two homologous integral cell- and mitochondrial membrane proteins (bcl-2 α and bcl-2 β isoforms). bcl-2 is a multi-lineal marker as the majority of B- cell lymphomas, a subset of T- cell lymphomas and many epithelial and mesenchymal neoplasms are or can be bcl-2 positive.⁸⁴ The main diagnostic use of bcl-2 in hematology is to distinguish between reactive lymph nodes with follicular hyperplasia exhibiting bcl-2 negative germinal centers and grade 1 follicular lymphoma with bcl-2 positive germinal centers.⁸⁴

C. Diagnostic antibodies for T- cell neoplasia: CD2, CD3, CD4, CD7, CD8 and TdT.⁸⁴

CD2 (LFA-2)		
Expression pattern: Membranous		
Main diagnostic use	Expression in other tumors	Expression in normal cells
T- cell lymphoma	Neoplastic mast cells (mastocytosis)	Thymocytes, mature peripheral T- cells, NK- cells
Positive control: Appendix / tonsil		

Diagnostic approach: CD2 is a transmembrane glycoprotein (E rosette receptor) and appears in the early stages of T- cell development. CD2 is an excellent marker for T- lymphocytes, T- cell lymphomas and the majority of NK neoplasia. CD2 is negative in B- lymphocytes with the exception of a small subset of thymic B- cells but negative in all B- cell lymphomas. CD2 is negative in normal mast cells and the CD2 positivity in mast cells is usually a criterion of malignancy.

CD3		
Expression pattern: Membranous		
Main diagnostic use	Expression in other tumors	Expression in normal cells
T- cell lymphomas	NK- lymphoma (cytoplasmic stain)	Thymocytes, peripheral T- cells, activated NK cells, Purkinje cells of cerebellum
Positive control: Appendix / tonsil		

Diagnostic approach: CD3 is a member of the immunoglobulin family functioning as a T- cell antigen receptor. CD3 is the most common used pan-T- cell marker expressed in the vast majority of T- cell lymphomas. CD3 reacts with many of the NK- lymphomas, but usually exhibiting a cytoplasmic stain pattern.

CD4		
Expression pattern: Membranous		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Mycosis fungoides, T- cell lymphomas	Histiocytic neoplasia, acute myeloid leukemia	Thymocytes, T- helper / inducer cells, macrophages, Langerhans cells, dendritic cells, granulocytes
Positive control: Appendix / tonsil		

Diagnostic approach: CD4 is a member of the immunoglobulin family and a marker for T- helper / inducer cells; the majority of thymocytes and lymphomas originated from these cells, which include the majority of peripheral T- cell lymphomas and cutaneous lymphomas, mainly mycosis fungoides.

Diagnostic pitfall: In immunohistochemistry and flow cytometry, CD4 must be used in a panel including CD3 and CD8 and CD19. CD4 can be also expressed in subtypes of acute myeloid leukemia and histiocytic neoplasia.

CD8		
Expression pattern: Membranous		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Subcutaneous panniculitis-like T- cell lymphoma	T- cell large granular lymphocytic leukemia, CLL, mantle cell lymphoma	Suppressor / cytotoxic T- cells and NK- cells
Positive control: Appendix / tonsil		

Diagnostic approach: CD8 is expressed in the suppressor / cytotoxic T- cells and also in a part of NK- cells. CD8 is a marker of many types of T/NK- cell lymphomas.

Diagnostic pitfall: CD8 is rarely expressed in some types of B- cell lymphomas and generally should be a part of panel with CD3, CD4 and CD20.⁷² Additionally, the expansion of the CD8 positive T- cell population is noted in lymph nodes-associated acute infectious mononucleosis.

CD7		
Expression pattern: Membranous		
Main diagnostic use	Expression in other tumors	Expression in normal cells
T- ALL and T- cell lymphomas	CML, immature myelomonocytic neoplasia, cholangiocellular carcinoma, pancreas carcinoma	Thymocytes, mature T- cells and NK cells, pre-B cells, monocytes, early myeloid cells
Positive control: Appendix / tonsil		

Diagnostic approach: CD7 is a member of the immunoglobulin family expressed in the majority of T- cell- and NK lymphomas.

Diagnostic pitfall: CD7 may be positive in a subset of AML and rarely in carcinomas such as pancreatic and bile duct carcinomas.⁷⁰

CD43		
Expression pattern: Membranous / cytoplasmic		
Main diagnostic use	expression in other tumors	Expression in normal cells
T- / NK cell lymphomas	B- ALL, Burkitt's lymphoma, mantle cell lymphoma, marginal zone lymphoma, granulocytic (myeloid) sarcoma, adenoid cystic carcinoma	Activated B- cells, T- cells, NK- cells, plasma cells, granulocytes
Positive control: Appendix / tonsil		

Diagnostic approach: CD43 is expressed on the membrane and in the cytoplasm of the T/NK- lymphocytes, cells of myeloid lineage, plasma cells and tumors originating from these cells.

Diagnostic pitfall: The expression of CD43 is found to be correlated with the expression of CD5 and expands not only to T- cell lymphomas, but also to many types of B- cell lymphomas, including chronic lymphocytic lymphoma, Burkitt's lymphoma, mantle cell lymphoma and marginal zone lymphoma.⁸⁴ Since normal B- lymphocytes lack the expression of CD43, CD43 positive B- lymphocytes are assumed to be neoplastic. Generally CD43 must be used in a panel with other more specific lymphoma markers.

Anaplastic lymphoma kinase (ALK, CD246)		
Expression pattern: Cytoplasmic / nuclear		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Anaplastic large cell lymphoma, inflammatory myofibroblastic tumor	Malignant peripheral nerve sheath tumor, rhabdomyosarcoma, neuroblastoma, Ewing's tumor / PNET, leiomyosarcoma	Glial cells, neurons, endothelial cells
Positive control: Anaplastic lymphoma / brain tissue		

Diagnostic approach: Anaplastic lymphoma kinase (ALK) is expressed during the embryogenesis and remains positive in glial cells of CNS. ALK is negative in normal lymphoid tissue but found to be positive in some lymphoma types, namely anaplastic large cell lymphoma, which is usually associated with the t(2; 5) or equivalent translocation.² ALK is also positive in the inflammatory myofibroblastic tumor which is also associated with the same translocation.⁸⁶

D. Diagnostic antibodies for NK- cell neoplasia: CD2, CD3, CD56, cytotoxic molecules (TIA-1, granzyme B, perforin) and EMA.⁸⁴

CD56 (N-CAM; NKH1)		
Expression pattern: Membranous		
Main diagnostic use	expression in other tumors	Expression in normal cells
NK- lymphomas, multiple myeloma, neuroendocrine tumors (small cell carcinoma, carcinoid and Merkel cell carcinoma), pheochromocytoma, neuroblastoma, medulloblastoma, astrocytomas, retinoblastoma, paraganglioma	Schwannoma, synovial sarcoma, embryonal and alveolar rhabdomyosarcoma, meningioma, melanoma, chordoma, epithelioid sarcoma	NK- cells, activated T- cells, cerebellum and brain cortex, neuromuscular junctions, neurons, intestinal ganglion cells, neuroendocrine tissue, thyroid follicular, epithelium, hepatocytes, epithelium of renal tubules, osteoblasts
Positive control: Brain tissue / intestinal ganglion cells		

Diagnostic approach: CD56 is a neural adhesion molecule involved in the development of neural cells and differentiation of neural tissue. CD56 is expressed in neuroectodermal cells, NK- cells, activated T- cells and related tumors.

Diagnostic pitfall: CD56 has a wide expression spectrum and it is found in subsets of CD4 and CD8 positive T- cells and plasma cells. CD56 is also expressed on the cells of multiple myeloma, whereas CD56 negative myeloma cases were found to have a poor prognosis. CD56 may be also expressed in some tumors with similar morphology such as embryonal rhabdomyosarcoma, neuroblastoma, small cell carcinoma and malignant melanoma, which must be considered in the differential diagnosis.^{47,70}

E. Antibodies for the diagnosis of Hodgkin’s lymphoma:

- **Diagnostic antibody panel for Classical Hodgkin’s lymphoma:** CD15, CD30, J- chain and fascin.^{73,74}
- **Diagnostic antibody panel for nodular lymphocyte predominant Hodgkin’s lymphoma:** CD19, CD20, PAX-5, J-chain, BOB.1, Oct-2 and EMA.⁷³

CD15		
Expression pattern: Membranous/ cytoplasmic		
Main diagnostic use	expression in other tumors	Expression in normal cells
Hodgkin’s lymphoma (Reed-Sternberg cells)	myeloid leukemia, adenocarcinoma, sweat- and sebaceous gland tumors, peripheral T- cell lymphoma, ALCL	Granulocytes and precursors (neutrophils and eosinophils), monocytes, activated B- and T- cells, proximal tubules of kidney, intestinal Paneth cells
Positive control: Appendix		

Diagnostic approach: CD15 (X hapten) is a frequently used marker for normal and neoplastic myeloid cells and monocytes. In combination with CD30, CD15 is commonly used as a marker for Reed-Sternberg cells of classical Hodgkin’s lymphoma. CD15 is also expressed on different carcinoma types but is negative in mesothelioma.

Diagnostic pitfall: In view of the fact that CD15 is expressed on the cells of different hematopoietic and non- hematopoietic neoplasms including adenocarcinomas, it is important to keep in mind the possible differential diagnoses and to support the final diagnosis by other more specific antibodies.

CD30		
Expression pattern: Membranous / cytoplasmic paranuclear		
Main diagnostic use	expression in other tumors	Expression in normal cells
Anaplastic large cell lymphoma, Reed-Sternberg cells in classic Hodgkin's lymphoma, primary mediastinal large B- cell lymphoma	Embryonal carcinoma, mesothelioma	Granulocytes, monocytes, activated B-, T- cells and NK- cells, decidua
Positive control: Embryonal carcinoma		

Diagnostic approach: CD30 (Ki-1) known as lymphocyte activation antigen, is normally expressed in activated B-, T- cells and NK- cells. One of the major utilities of CD30 is to highlight Reed-Sternberg cells of classical Hodgkin's lymphoma, anaplastic large cell lymphoma and primary mediastinal large B- cell lymphoma.

Diagnostic pitfall: CD30 positive cells may be found in some other lymphoma types and also in normal reactive lymph nodes, consequently not all CD30 positive cells are Hodgkin's cells. It is also important to keep in mind that CD30 is also positive in some types of primary and metastatic germ cell tumors.

Fascin (actin bundling protein; p55)		
Expression pattern: Membranous / cytoplasmic		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Reed-Sternberg cells in classic Hodgkin's lymphoma, anaplastic large cell lymphoma, follicular- and interdigitating dendritic cell tumors	Adenocarcinoma of breast, colon, biliary tract, pancreas, lung, ovary and skin; papillary transitional cell carcinoma of bladder, diffuse large B- cell lymphoma,	Interdigitating and follicular dendritic cells, endothelial cells, EBV infected B- lymphocytes
Positive control: Lymph node		

Diagnostic approach: Fascin is an actin binding protein, normally expressed in interdigitating and follicular dendritic cells and variably in endothelial cells. Fascin is a good marker for Reed-Sternberg cells of classical Hodgkin’s lymphoma. It is also expressed on the membrane of anaplastic large cell lymphoma and subtypes of diffuse large B- cell lymphoma. Fascin is constantly negative in normal epithelium but positive in many types of neoplastic epithelium. This phenomenon may be used for the differentiation between hyperplastic and neoplastic urothelium.

Diagnostic pitfall: As a consequence of the wide expression spectrum of fascin, many of the above mentioned differential diagnoses must be considered in the interpretation of the fascin immunostain. In addition to Reed Sternberg cells, fascin positive cells in lymph nodes could be activated B- lymphocytes, cells of diffuse large B- cell lymphoma or even disseminated cells of metastatic adenocarcinoma.

F. Diagnostic antibody panel for plasma cell neoplasia: CD38, CD138, VS38c, MUM-1, κ and λ light chains.

CD38		
Expression pattern: Membranous		
Main diagnostic use	expression in other tumors	Expression in normal cells
Plasma cell neoplasia, plasmablastic lymphoma	Pre-T- ALL, primary effusion lymphoma, subtypes of B- cell lymphoma	Plasma cells, erythroid and myeloid precursors, early B- and T- cells, NK- cells, pancreatic islets, neuronal tissue
Positive control: Appendix		

Diagnostic approach: CD38 is a transmembrane glycoprotein expressed in different maturation stages of B- and T- lymphocytes, plasma cells and myeloid cells.⁷¹ CD38 is one of the markers used in the diagnostic panel for multiple myeloma.

Diagnostic pitfall: Because of the wide multilineal expression-spectrum of CD38, it necessary to confirm the plasma cell nature of the neoplasia by other plasma cell markers and the CD45 negativity.

CD138 (Syndecan-1)		
Expression pattern: Membranous / cytoplasmic		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Plasma cell tumors (myeloma, plasmacytoma)	Primary effusion lymphoma, thyroid carcinoma, breast carcinoma, pulmonary carcinoma, squamous cell carcinoma, neuroendocrine tumors, adrenal cortical tumors, keratoacanthoma	B- cell precursors, plasma cells, stratified squamous epithelium
Positive control: Tonsil / squamous epithelium		

Diagnostic approach: CD 138 (syndecan-1) is a transmembrane antigen and one of the 4 members of the syndecan family. The expression of CD138 is not restricted to different stages of B-lymphocytes and plasma cells but also found in different types of epithelial and mesenchymal cells; however CD138 is one of the important markers for plasma cell neoplasia.

Diagnostic pitfall: CD138 is widely used as a marker for plasma cells and plasma cell neoplasia but the expression of CD138 by the cells of squamous epithelium, squamous cell carcinoma and even adenocarcinoma of different origin makes it necessary to exclude the possibility of undifferentiated carcinoma using a pan-cytokeratin antibody but not EMA, which may be positive in plasma cell disorders.⁷⁰ The expression profile of κ and λ light chains is also important to determine the clonality of plasma cell proliferation.

MUM-1 (multiple myeloma oncogene 1/IRF4)		
Expression pattern: Nuclear / cytoplasmic		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Plasma cell tumors	DLBCL, malignant melanoma	B- cells (centrocytes), plasma cells, activated T-cells
Positive control: Appendix		

Diagnostic approach: The MUM1 protein is a lymphocyte-specific transcriptional activator expressed in the final differentiation stage of intra-germinal center B- cells. MUM1 is a marker for post-germinal center B- cells, plasma cells and subset of T- cells and related neoplasms. Because of

the multilineal expression of the MUM1 protein⁷⁶, the immunostaining results must be carefully interpreted in combination with more specific antibody panel to exclude other possible differential diagnoses.

VS38c (plasma cell marker)		
Expression pattern: Cytoplasmic		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Plasma cell neoplasia (myeloma, plasmacytoma), lymphoma with plasmacytic differentiation	Rare carcinomas, melanoma, clear cell sarcoma of soft tissue, neuroendocrine tumors	Plasma cells & plasmablasts, B- immunoblasts, epithelial cells (mucous glands, pancreatic epithelium, secretory breast cells, thyroid follicles), melanocytes, osteoblasts
Positive control: Appendix		

Diagnostic approach: VS38c is a sensitive screening marker for plasma cells and plasma cell differentiation expressed intracytoplasmic on the endoplasmic reticulum. The expression of VS38c is found in plasma cells, plasmablasts, lymphoplasmacytoid cells and B- immunoblasts and related neoplasia.

Diagnostic pitfall: Despite the specificity and high sensitivity of VS38c to normal and neoplastic plasma cell, it is always important to keep in mind that other cell types such as melanocytic and neuroendocrine tumors may be positive for this marker.⁷⁵ Paratrabeular osteoblasts in trephine biopsies are also positive for VS38c.

5.13 . Antibodies for the diagnosis of myeloid neoplasia

A. Diagnostic antibody panel for myeloid neoplasia: CD13, CD33, CD15 and MPO.

Myeloperoxidase (MPO)		
Expression pattern: Cytoplasmic		
Main diagnostic use	Expression in other tumors	Expression in normal cells
AML	Granulocytic sarcoma	myeloid cells, monocytes
Positive control: Bone marrow		

Diagnostic approach: Myeloperoxidase (MPO) is one of the main lysosomal enzymes in myeloid cells. MPO positivity is diagnostic for myeloid origin and constantly negative in normal and neoplastic lymphoid tissue.

CD15 is a further important marker for the myeloid lineage listed in a previous section.

B. Diagnostic antibody panel for mast cell tumors: Mast cell tryptase, CD117, CD2 and CD33.^{77,78}

Mast cell tryptase		
Expression pattern: Cytoplasmic		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Mast cell tumors		Mast cells
Positive control: Appendix		

Diagnostic approach: Tryptase is one of the mediators of inflammation found in mast cells. Antibodies to tryptase are used as specific markers for mast cells but cannot discriminate between normal and neoplastic mast cells.

CD2: CD2 is previously listed in the lymphoma section, normally expressed in different stages of T-cell development. CD2 is negative in B- lymphocyte, B- cell lymphomas and normal mast cells, but the CD2 positivity in mast cells indicates a neoplastic nature of these cells.⁷⁸

5.14 . Antibodies for the diagnosis of histiocytic and dendritic cell tumors

Diagnostic antibody panel for histiocytic and dendritic cell tumors: CD1a, CD21, CD35, CD68, fascin, Podoplanin and S100.

CD1a		
Expression pattern: Membranous		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Langerhans cell histiocytosis	Myeloid leukemia, mycosis fungoides, cutaneous T- cell lymphomas, T- ALL	Cortical thymocytes, Langerhans cells, immature dendritic cells
Positive control: Skin		

Diagnostic approach: CD1a is a surface antigen of cortical thymocytes and dendritic cells in addition to Langerhans cells. CD1a is a specific marker for normal and neoplastic Langerhans cells but constantly negative in histiocytic- follicular dendritic- and interdigitating cell tumors. CD1a is also expressed in some types of T- cell lymphoma, chiefly cutaneous T- cell lymphoma.

CD68		
Expression pattern: Cytoplasmic		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Histiocytic tumors, dendritic cell tumors, AML (FAB-M4/M5), giant cell tumors	Fibrous histiocytoma, nodular fasciitis, villonodular synovitis, granular cell tumor, inflammatory myofibroblastic tumor, mast cell disease, hairy cell leukemia	Macrophage, monocytes, osteoclasts, Kupffer cells, mast cells, synovial cells, microglia, dendritic cells, fibroblasts, Langerhans cells, myeloid cells, CD34+ progenitor cells, neutrophils, B- and T- cells
Positive control: Appendix		

Diagnostic approach: CD68 is a cytoplasmic glycoprotein involved in the regulation of phagocytic activity of the macrophages. CD68 is a widely used marker for histiocytes and histiocytic tumors but lacks the specificity to these cells.⁸⁶

Diagnostic pitfall: CD68 has a wide expression range and may be positive in different hematologic disease of B- cell, T- cell, NK and myeloid lineage.

CD21		
Expression pattern: Membranous		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Follicular dendritic cell sarcoma	Hairy cell leukemia, mantle cell and marginal zone lymphoma	Follicular dendritic cells, mature B- cells, pharyngeal and cervical epithelial cells
Positive control: Lymph node		

Diagnostic approach: CD21 is a C3d receptor on the B- lymphocytes, also expressed by follicular dendritic cells. CD21, CD35 and podoplanin are very helpful markers for follicular dendritic cell tumors (sarcoma) but CD21 is also positive in a subset of B- cell lymphoma and rarely T- cell lymphoma.^{79,80,81,82} CD21 is usually negative in histiocytic-, Langerhans cell- and interdigitating cell tumors.

Fascin: Fascin is an actin-binding protein listed as a marker for Reed-Sternberg cells. Fascin is strongly expressed in normal and neoplastic interdigitating and follicular dendritic cells.⁸⁰

5.15 . Antibodies for the diagnosis of malignant melanoma

Malignant melanoma has a variable morphologic appearance and the diagnosis must be based on the morphology, immunoprofile and clinical data. In metastatic tumors with ambiguous morphology it is always advisable to rule out melanoma.

Diagnostic antibody panel for malignant melanoma: HMB45, MART-1, tyrosinase, WT-1, S100 and CD63 (NK-C3).

HMB-45		
Expression pattern: Cytoplasmic		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Malignant melanoma, Spitz and cellular blue nevi, clear cell sarcoma, PEComa	Angiomyolipoma, sugar tumor of lung, pheochromocytoma,	Junctional melanocytes / some nevus cells, mononuclear cells
Diagnostic approach: Melanoma		

Diagnostic approach: HMB45 is a melanosomal glycoprotein (gp100). Antibodies to HMB45 stain malignant melanoma and tumors with melanocytic differentiation including clear cell sarcoma, Spitz and blue nevi, whereas intradermal nevi and mature melanocytes are negative for HMB45.

Diagnostic pitfall: About 10% of malignant melanoma (more frequently desmoplastic and spindle cell melanomas) lacks the HMB45 expression. The use of an antibody cocktail of different anti-melanoma markers (usually HMB45, MART-1 and tyrosinase) will markedly increase the sensitivity. Additionally, tumors with similar morphology such as pheochromocytoma and clear cell tumor (sugar tumor) of the lung may be positive for HMB45 but these are usually negative for tyrosinase.

Tyrosinase		
Expression pattern: Cytoplasmic		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Malignant melanoma	Clear cell sarcoma, benign melanocytic lesions	Melanocytes
Positive control: Skin / melanoma		

Diagnostic approach: Tyrosinase is an enzyme involved in the syntheses of melanin. Antibodies to tyrosinase are very specific melanoma markers. Because of its high specificity, tyrosinase is frequently used with other antibodies in one mixture as pan-melanoma marker. This pan-melanoma cocktail gives good results in the diagnosis of epithelioid-, desmoplastic- and spindle cell melanomas and is effective in the detection of micrometastases in sentinel lymph nodes.

MART-1 (melan A)		
Expression pattern: Cytoplasmic		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Melanoma, adrenal cortical tumors, sex cord-stromal tumors	Angiomyolipoma, osteosarcoma,	Adrenal cortex, melanocytes, brain tissue, granulosa and theca cells, Leydig cells
Positive control: Adrenal cortex		

Diagnostic approach: MART-1 (also known as melan A) is a melanoma antigen recognized by cytotoxic T- lymphocytes normally expressed in melanocytes of skin and retina and related tumors.

Diagnostic pitfall: MART-1 is one of the specific melanoma markers, however it is also an important diagnostic marker for other tumors as mentioned previously such as adrenocortical and sex cord-stromal tumors. We recommend using MART-1 as a screening antibody and to confirm the diagnosis by 1-2 further antibodies.

Wilms Tumor protein (WT-1): WT-1 is another marker for malignant melanoma listen in the mesothelioma section.⁸³ Similar to HMB45, WT-1 is a helpful marker to discriminate between malignant and benign melanocytic lesions.

5.16 . Antibodies for the diagnosis of muscle tumors

A. **Diagnostic antibody panel for skeletal muscle tumors:** Desmin, myoglobin, myogenin and MyoD1.^{85,86}

Desmin		
Expression pattern: Cytoplasmic		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Rhabdomyosarcoma and rhabdomyoma, smooth muscle tumors	Desmoplastic small round cell tumor, alveolar soft part sarcoma, malignant rhabdoid tumor, tenosynovial giant-cell tumor	Smooth and striated muscle, myoblasts and myofibroblasts, mesothelial cells, endometrium
Positive control: Appendix		

Diagnostic approach: Desmin is an intermediate filament protein present in both skeletal- and smooth muscle cells. Desmin is an important diagnostic marker for myogenic tumors and myogenic differentiation, whereas myoepithelial cells are negative for desmin.

Diagnostic pitfall: Desmin is found in other tumors with similar morphology to rhabdomyosarcoma types such as desmoplastic small round cell tumor and alveolar soft part sarcoma; hence the diagnostic panel for rhabdomyosarcoma must include at least one of the antibodies to myogenic transcriptional regulatory proteins (Myogenin, Myo D-1 or Myf-3). Markers for smooth muscle differentiation must be also included. It is noteworthy that mesothelioma (mainly sarcomatous mesothelioma), but rarely carcinomas, can show positivity to desmin, which makes it necessary in doubtful cases to determine the cytokeratin profile.

Myoglobin		
Expression pattern: Cytoplasmic		
Main diagnostic use	Expression in other tumors	Expression in normal cells
tumors with skeletal muscle differentiation / rhabdomyosarcoma		Striated muscle
Positive control: Skeletal muscle		

Diagnostic approach: Myoglobin is an oxygen-binding protein. Myoglobin is expressed in skeletal and cardiac muscle and adult type skeletal muscle tumors. Embryonal muscle tumors and smooth muscle tumors lack the expression of myoglobin.

Myogenin and Myo D1		
Expression pattern: Nuclear		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Rhabdomyosarcoma	Wilms tumor	Fetal muscle, myoblasts
Positive control: Rhabdomyosarcoma / fetal muscle		

Diagnostic approach: Both Myogenin (Myf-4) and Myo D1 in addition to Myf-3 and Myf-5 are myogenic transcriptional regulatory proteins involved in the regulation of early skeletal muscle differentiation and maintenance of myogenic program. Antibodies to myogenin and Myo D1 are specific markers for all rhabdomyosarcoma types.^{87,88}

Diagnostic pitfall: Both markers can be positive in myoblasts found in regenerative and atrophic muscle lesions⁸⁹. Desmoid tumors and infantile fibrosarcoma can also show positivity to myogenin and MyoD1. In the interpretation of myogenin and Myo D1 stains, only nuclear stain can be considered positive, other stain types (cytoplasmic or membranous) are non-diagnostic artifacts.

PAX-5: PAX-5 was discussed as a B- cell marker and marker for some neuroendocrine carcinomas. PAX-5 is also a specific marker for alveolar rhabdomyosarcoma but it is constantly negative in embryonal type rhabdomyosarcoma.⁶⁸

B. Diagnostic antibody panel for smooth muscle tumors: Desmin, sm-actin, h-caldesmon, calponin and steroid hormones.⁹⁰

Smooth muscle Actin (sm-actin)		
Expression pattern: Cytoplasmic		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Smooth muscle tumors	Myoepithelial and myofibroblastic tumors GIST, endometrial stromal sarcoma	Smooth muscle cells, myoepithelial cells, myofibroblasts
Positive control: Appendix		

Diagnostic approach: Actins are a group contractile microfilaments. Smooth muscle actin (sm-actin) is one of various known actins and antibodies to sm-actin react with α smooth muscle actin and stain smooth muscle cells in addition to myoepithelial and myofibroblastic cells. Clone 1A4 is a widely used antibody to sm-actin, effective for the diagnosis of smooth muscle-, myoepithelial- and myofibroblastic lesions.⁸⁶

Diagnostic pitfall: sm-actin can be positive in some tumors with a similar morphology other than smooth muscle tumors, including endometrial stromal tumors, synovial sarcoma, GIST and sarcomatous mesothelioma.

h-Caldesmon		
Expression pattern: Cytoplasmic		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Smooth muscle tumors	Glomus tumors, GIST, myoepithelial and myofibroblastic tumors epithelioid mesothelioma	Smooth muscle cells, myoepithelial cells
Positive control: Appendix		

Diagnostic approach: h-Caldesmon is a calcium binding protein involved in the regulation of smooth muscle contraction. In non-smooth muscle tumors the expression spectrum of caldesmon is narrower than that of sm-actin.

Calponin (basic)		
Expression pattern: Cytoplasmic		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Smooth muscle tumors	Myoepithelial and myofibroblastic tumors	Smooth muscle, myoepithelial cells,
Positive control: Appendix		

Diagnostic approach: Calponin (basic type) is an actin binding protein involved in the regulation of smooth muscle contraction. The expression spectrum of Calponin is similar to that of h-caldesmon.

5.17 . Antibodies for the diagnosis of vascular tumors

Diagnostic antibody panel for vascular tumors: CD31, CD34, factor VIII, CD105, CD141, podoplanin and Fli-1.⁹²

CD31 (PECAM-1)		
Expression pattern: Membranous		
Main diagnostic use	expression in other tumors	Expression in normal cells
Vascular tumors	Plasmacytoma, Langerhans cell histiocytosis, granulocytic sarcoma, Ewing's sarcoma	Endothelial cells, megakaryocytes / platelets, macrophages / monocytes, Kupffer cells, osteoclasts, granulocytes, mantle zone B-cells, T/NK- cells and plasma cells
Positive control: Appendix		

Diagnostic approach: CD31 known as PECAM-1 (platelet endothelial cell adhesion molecule-1) is a member of the immunoglobulin family normally expressed on endothelial cell junctions and on the surface of platelets. CD31 is a sensitive marker for blood vessels and vascular tumors.^{92,93}

CD34		
Expression pattern: Membranous		
Main diagnostic use	expression in other tumors	Expression in normal cells
Vascular tumors, Kaposi's sarcoma, GIST, dermatofibrosarcoma protuberans, solitary fibrous tumor, epithelioid sarcoma, AML (M0), granulocytic sarcoma, neurofibroma, liposarcoma	Pre B-ALL, AML (M7), alveolar soft part sarcoma, congenital and infantile fibrosarcoma, inflammatory fibrous polyp of GIT, breast fibroadenoma, giant cell fibroblastoma, juxtaglomerular cell tumor, superficial acral fibromyxoma	Hematopoietic progenitor cells, B- and T- lymphocyte precursors, endothelial cells, hepatic sinusoidal cells, interstitial cells of Cajal, endometrial stroma, fibroblasts
Positive control: Appendix		

Diagnostic approach: CD34 is a cell surface adhesion glycoprotein expressed on the surface of precursor hematopoietic cells, a subset of mesenchymal stem cells and endothelial cells and a large number of tumors originated from these cells. CD34 is a widely used marker to highlight blood vessels and vascular tumors but it is less specific than CD31.^{92,93} CD34 is also an important marker for other tumors such as dermatofibrosarcoma protuberans and GIST.

Diagnostic pitfall: Because of its broad expression spectrum, CD34 must be used as a screening marker supported by a panel of more specific antibodies.⁸⁶

Factor VIII (von Willebrand factor)		
Expression pattern: Cytoplasmic		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Vascular tumors		Endothelial cells, platelets and megakaryocytes, mast cells
Positive control: Appendix		

Diagnostic approach: Factor VIII is a glycoprotein synthesized by endothelial cells and megakaryocytes. Factor VIII is a specific marker for blood vessels and vascular tumors but it has a low sensitivity in poorly differentiated vascular tumors.

Podoplanin (D2-40)		
Expression pattern: Membranous/ cytoplasmic		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Lymphangioma and other tumors of lymphatic vessels mesothelioma, adenomatoid tumor	Vascular tumors, skin adnexal carcinomas, germ cell tumors, Kaposi's sarcomas, follicular dendritic cells tumors, schwannoma, meningioma, GIST, synovial sarcoma, leiomyosarcoma, desmoid, malignant peripheral nerve sheath tumor, chondrosarcoma	Lymphatic endothelium, mesothelial cells, granulosa cells, adrenal cortex, follicular dendritic cells, osteocytes, ependymal cells
Positive control: Appendix		

Diagnostic approach: Podoplanin is a mucoprotein expressed on the membrane of several cell types, mainly lymphatic endothelium and mesothelial cells.^{92,93} Podoplanin is very helpful in highlighting the lymphatic vessels and is good marker for tumors of lymphatic vessels and mesothelioma.

Diagnostic pitfall: Podoplanin has a broad expression spectrum, can be expressed in various tumors with ambiguous morphology such as leiomyosarcoma, desmoid and peripheral nerve sheath tumors, and must be used as a part of a panel with other more specific antibodies.⁵⁷

5.18 . Antibodies for the diagnosis of lipomatous tumors

Diagnostic antibody panel for lipomatous tumors: S100, CD34, MDM2 and CDK4.

MDM2		
Expression pattern: Nuclear / cytoplasmic		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Liposarcoma	Clear cell sarcoma, desmoplastic small round cell tumor, angiosarcoma, Kaposi's sarcoma, epithelioid sarcoma, embryonal rhabdomyosarcoma, leiomyosarcoma, MPNST, adrenal oncocytoma,	
Positive control: Liposarcoma		

Diagnostic approach: MDM2 (Murine Double Minute 2) is a nuclear phosphoprotein that interacts with p53 affecting the cell cycle and apoptosis. MDM2 is over-expressed in many tumors while the main diagnostic use is to differentiate between benign adipocytic tumors and well differentiated liposarcoma.^{94,95,96}

Diagnostic pitfall: As mentioned above, MDM2 is positive in many sarcoma types, which must be considered in the differential diagnosis. It should also be considered that the MDM2 antibody clone SMP14 shows cross reactivity with some cytokeratins including the cytokeratins 6, 14 and 16, which label squamous epithelium and squamous cell carcinoma.

5.19 . Antibodies for the diagnosis of peripheral nerve- and nerve sheet tumors

Diagnostic antibody panel for peripheral nerve- and nerve sheet tumors: S100, CD56, protein gene product 9.5, myelin basic protein, glial fibrillary acidic protein (GFAP), neurofilaments.

Myelin basic protein		
Expression pattern: Cytoplasmic		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Neurogenic sarcoma, neuroma, neurofibroma, ganglioneuroma	Granular cell tumor	Cells of white matter of central and peripheral nervous systems
Positive control: Brain tissue		

Diagnostic approach: Myelin basic protein (MBP) is produced by oligodendrocytes and is localized in myelin surrounding nerve fibers in both the central nervous and the peripheral nervous systems. Antibodies to MBP are used as a marker for neuroma, neurofibroma and neurogenic sarcoma but are negative in other spindle cell tumors.

Neurofilaments		
Expression pattern: Cytoplasmic		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Medulloblastoma, retinoblastoma, neuroblastoma, ganglioglioma	Merkel cell tumor, pancreatic endocrine neoplasms, carcinoid, small cell carcinoma, parathyroid tumors, pheochromocytoma	Neuronal cells
Positive control: Brain tissue		

Diagnostic approach: Neurofilaments are the main cytoskeletal element in nerve axons and dendrites of both central and peripheral nervous system. Neurofilaments are good markers for tumors of neurons and ganglion cells and for tumors with neuronal differentiation.

Diagnostic pitfall: The expression of the neurofilaments is reported in rare cases of non-neurogenic tumors such as rhabdomyosarcoma and epithelioid sarcoma and some carcinoma types.

Protein gene product 9.5 (PGP 9.5)		
Expression pattern: Cytoplasmic		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Malignant nerve sheet tumor, paraganglioma	Carcinoid tumors, Merkel cell carcinoma, Granular cell tumor, atrial myxoma	Neurons and nerve fibers, neuroendocrine cells, renal tubular epithelium, spermatogonia, Leydig cells
Positive control: Brain tissue		

Diagnostic approach: Protein gene product 9.5 (PGP 9.5) is a neuron specific protein in the central and peripheral nervous systems. Antibodies to PGP9.5 are good markers to highlight neuronal and neuroendocrine tumors.

Diagnostic pitfall: PGP9.5 has a low specificity, is reported to be expressed in a number of non-neuronal tumors and must be supported by other more specific markers.⁹⁷

Glial fibrillary acidic protein (GFAP)		
Expression pattern: Cytoplasmic		
Main diagnostic use	Expression in other tumors	Expression in normal cells
CNS tumors (astrocytoma, glioblastoma, oligodendroglioma, medulloblastoma, ependymoma), retinoblastoma, neurolemoma, neurothekoma, MPNST	Salivary gland tumors (myoepithelial adenoma/carcinoma, basal cell adenoma / carcinoma, pleomorphic adenoma)	Astrocytes, oligodendrocytes, subset of CNS ependymal cells, Schwann's cells, Kupffer cells, chondrocytes
Positive control: Brain tissue		

Diagnostic approach: Glial fibrillary acidic protein (GFAP) is an intermediate filament protein mainly expressed in normal and neoplastic glial cells, astrocytes, ependymal cells in addition to Schwann cells. GFAP is an important marker to discriminate between primary brain- and metastatic tumors. GFAP is also expressed in different types of salivary gland tumors.

5.20 .Antibodies for the diagnosis of Ewing's sarcoma / primitive neuroectodermal tumors (PNET)

Diagnostic antibody panel for Ewing's / primitive neuroectodermal tumors: CD99, Fli-1, chromogranin and synaptophysin.

CD99 (MIC2)		
Expression pattern: Membranous / cytoplasmic		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Ewing's sarcoma / PNET, T- and B- ALL, solitary fibrous tumor	T- cell lymphoma, anaplastic large cell lymphoma, AML, GIST, metaplastic carcinoma of the breast, hepatocellular carcinoma, thymoma, ovarian sex cord stromal tumors, synovial sarcoma, Merkel cell carcinoma, endocrine and neuroendocrine tumors, desmoplastic small round cell tumor, melanoma, neuroblastoma, ependymoma, mesenchymal chondrosarcoma, extrarenal malignant rhabdoid tumor, rhabdomyosarcoma, meningeal hemangiopericytoma	Cortical thymic lymphocytes, ovarian granulosa cells, pancreatic islets, Sertoli cells, endothelial cells, T- cells & activated B- cells, ependymal cells
Positive control: PNET		

Diagnostic approach: CD99 also known as MIC2 is a cell surface glycoprotein involved in the differentiation of primitive neuroectodermal cells and T- cells. CD99 has a broad multi-lineal expression spectrum in a large number of normal and neoplastic cells. CD99 is widely used as a marker for Ewing's sarcoma / PNET family exhibiting a membranous stain, while a cytoplasmic stain can be noted in other tumor types. CD99 is constantly negative in neuroblastoma.

Diagnostic pitfall: As listed in the table above, CD99 has a very wide expression spectrum and low specificity, consequently CD99 should never be used as a single marker for tumor diagnosis,

especially in tumors with similar morphology such as PNET and ALL.^{86,98} A panel of more specific antibodies must be always used to confirm the diagnosis.

Fli-1		
Expression pattern: Nuclear		
Main diagnostic use	Expression in other tumors	Expression in normal cells
Ewing's sarcoma, vascular tumors	Lymphoblastic lymphoma	Endothelial cells, T-lymphocytes
Positive control: Endothelial cells		

Diagnostic approach: Fli-1 gene (friend leukemia virus integration site 1), is a member of the ETS proto-oncogene family functioning as a transcriptional activator. The Fli-1 gene is involved in t(11; 22)(q24; q12) translocation, the most common and the most specific molecular marker for Ewing's sarcoma / PNET family; it is found in more than 90% of the cases. Available monoclonal and polyclonal antibodies to Fli-1 found to be of high specificity to the PNET family.

Diagnostic pitfall: The expression of the Fli-1 transcription factor is not restricted to PNETs but also found in vascular tumors and rarely in some lymphoma types which may have a similar morphology to PNET.⁹⁹ CD99 and Fli-1 positivity in correlation with the clinical data is required for the precise diagnosis of this tumor family.

6. Results

In our daily practice, immunohistochemistry is a very powerful tool essential for diagnostic tumor histopathology. In this study based on the available clinical data, conventional tumor morphology and immunoprofile 2691 out of the 2907 tumors examined were successfully characterized. It was not only possible to differentiate between carcinoma, soft tissue tumor (sarcoma), lymphoma or hematological neoplasia and primary tumor of the central nervous system but we were able to give sufficient histogenetic characterization required for adequate tumor therapy. 198 additional tumors were examined only to determine the sensitivity of the tumor to therapeutic agents (estrogen- and progesterone receptors and HER-2). 18 cases (less than 0.7% of the total cases received for tumor diagnoses) remain unclear and were sent to highly specialized pathology centers for a second opinion or further investigation.

Listed below are the results of the tumors studied along with summarized diagnostic approach. To determine the phenotype of unknown tumors, the first screening panel (page 16) was used as a guide, usually followed by panels of more specific antibodies. To determine the type and the origin of carcinoma, we analyzed the cytokeratin profile and the expression of different tissue specific markers with the following results:

- The differentiation between malignant epithelial tumors (carcinomas) and tumors of other differentiation lines was possible in 2170 cases based on the clinical data, morphology and immunophenotype, mainly the cytokeratin profile.
- Squamous cell carcinoma was confirmed in 480 cases based on the morphology and the expression of the high molecular weight cytokeratins (cytokeratins 5/6/10/14). The main diagnostic difficulty was to determine the origin of metastatic carcinoma, as squamous cell carcinoma usually lacks any other specific markers with the exception of p16, which favors the uterine cervix as possible origin of primary squamous cell carcinoma. Correlation with the other clinical and radiological data was essential for further management of tumors.
- The diagnosis of adenocarcinoma was confirmed in 1690 cases based on the morphology and the expression of cytokeratin 7 and / or cytokeratin 20 in addition to the expression of the carcinoembryonic antigen (CEA). To determine the origin of metastatic adenocarcinoma, the following tissue and organ specific markers were used:

- TTF-1 and / or surfactant proteins were used to confirm the pulmonary origin of the carcinoma. In cases of single TTF-1 expression, the thyroid origin was ruled out by screening for thyroglobulin or calcitonin expression.
 - CDX-2 was used as an important criterion for carcinomas of gastrointestinal tract origin. Diagnostic pitfalls mentioned in the description of CDX-2 were also considered in metastatic tumors. The expression profile of cytokeratins 7 and 20 was helpful to determine the location of the adenocarcinoma in the gastrointestinal tract. Ovarian surface epithelial-stromal tumors with similar morphology and immunoprofile were also discussed in the differential diagnosis. In 2 cases, a certain differentiation between metastatic mucinous ovarian carcinoma and colorectal adenocarcinoma was not possible.
 - The expression of mammaglobin, GCDFP15 and steroid receptors was considered an important criterion for breast origin. Based on this profile 15 lymph node-, 8 lung- and pleura- and 3 liver metastases were diagnosed as metastatic breast carcinoma. In the case of single estrogen receptor-expression, other differential diagnoses, mainly tumors of the female genital tract and hepatocellular carcinoma were considered.
- Using specific cytokeratins, 18 salivary gland tumors were examined to label the epithelial and myoepithelial cell components or to rule out metastatic carcinoma.
 - 24 oral biopsies were examined. In 15 cases the epithelial cells were labeled to highlight possible microinvasion, in 6 cases to exclude malignant melanoma and in 3 cases to confirm neurofibroma.
 - The diagnosis of primary thyroid carcinoma was confirmed by the TTF-1 expression, whereas the thyroglobulin expression supported the diagnosis of thyroid-follicle-cell origin in 7 metastatic carcinomas. Cytokeratin 19 was used to support the differentiation between the variants of papillary thyroid carcinoma and follicular lesions. The calcitonin expression confirms the C- cell origin in 1 metastatic carcinoma diagnosed as medullary thyroid carcinoma. Another 3 cases of localized medullary thyroid carcinomas were confirmed by the TTF-1, calcitonin and CEA positivity. The parathyroid origin of 4 benign tumors (parathyroid adenomas) was confirmed by the expression of the parathyroid hormone.
 - 22 out of the 49 mediastinal biopsies examined were diagnosed as lymphoma of different types, 17 metastatic carcinoma of different primaries, 5 cases as thymoma or thymic carcinoma, 3 cases as germ cell tumors and 2 as benign mesenchymal tumors.

- Seven classical, 4 atypical carcinoid tumors and 23 small carcinomas of the lung were confirmed based on the morphology and the expression of CD56, chromogranin and TTF-1 in addition to the proliferation index estimated by Ki-67 staining. Non-pulmonary TTF-1 positive neuroendocrine carcinomas (small cell carcinomas) were also considered in the differential diagnosis.
- 18 neuroendocrine tumors of the gastrointestinal tract were confirmed based on the expression chromogranin and CDX-2. Four pancreatic neuroendocrine tumors were diagnosed based on the expression of neuroendocrine markers and specific hormones.
- Four cases of hepatocellular carcinoma were confirmed based on the alpha-fetoprotein (AFP) expression and CD34 staining pattern of the sinusoidal cells. No AFP negative hepatocellular carcinomas were recorded. Cholangiocellular carcinoma was diagnosed in 3 cases based on the expression of cytokeratin 7, CEA and absence of AFP expression. 57 liver metastases of GIT-, pulmonary-, breast carcinomas, gastrointestinal stroma tumors (GIST) and melanoma were diagnosed based on the morphology and characteristic immunoprofile. The GIST cases were examined for the expression of CD117 for specific therapy.
- Metastases of renal cell carcinoma were confirmed in 4 cases. The diagnosis was supported by the expression of CD10 and renal cell carcinoma marker (RCC). This profile was also useful to discriminate between renal cell carcinoma and transitional carcinoma.
- The diagnosis of transitional cell carcinoma of the urinary tract was made based on the co-expression of cytokeratins 5, cytokeratin 7, CD141 and uroplakin 3. Uroplakin and Cytokeratin 5 were important to discriminate between transitional cell carcinoma and renal cell carcinoma in 2 poorly differentiated renal tumors. Based on the morphology, clinical data and the single expression of cytokeratin 7, the diagnosis of primary urinary bladder adenocarcinoma was confirmed in 3 cases.
- For the diagnosis of metastatic prostatic carcinoma, prostate specific antigen (PSA), prostatic acid phosphatase (PAP) and androgen receptors were excellent markers. Using the combination of 2 or 3 of the markers mentioned we were able to confirm the prostatic origin of metastatic carcinoma in 8 cases. To confirm the diagnosis of local prostatic carcinoma, the staining of the myoepithelial cells with high molecular cytokeratins or p63 gave superior results in all cases examined. The double stain with AMACR made the diagnosis more convenient. Using this approach we were able to confirm the diagnosis in 116 cases and to rule out the diagnosis in 79 cases.

- Four lymph node metastases of testicular seminoma were confirmed based on the expression of CD117, OCT3/4 and placental alkaline phosphatase. To screen for other components of germ cell tumors, CD30 and AFP were used. According to the morphology and the characteristic immunoprofile 21 pure seminomas, 6 cases of intratubular germ cell neoplasia and 8 mixed germ cell tumors in addition to 1 Leydig cell tumor were diagnosed.
- 11 lymph node- and organ metastases of malignant melanoma were diagnosed based on the expression of 1 or 2 melanoma specific markers.
- The diagnosis of 5 pleural and 2 peritoneal mesotheliomas were confirmed based on the expression of 3 mesothelial markers and the absence of any other tissue specific marker such as CEA, TTF-1, CDX-2, steroid receptors or mammaglobin. 27 pleura- and peritoneal carcinosis of different primaries were diagnosed based on the expression of the specific markers.
- 21 out of the 25 examined retroperitoneal tumors were diagnosed as lymphoma of different types, 2 cases as paraganglioma and 2 cases as metastatic seminoma.
- 172 lymph nodes were examined for lymphoma diagnoses or for lymphoma typing using B-, T- and Hodgkin cell markers in addition to Ki-67. B- cell lymphoma of different types was diagnosed in 208 cases. T- cell lymphoma of different types was diagnosed in 19 cases and the diagnoses were usually confirmed by gene rearrangement analysis. Hodgkin's lymphoma was diagnosed in 36 cases. Eight lymph nodes were examined for the diagnosis of histiocytic and follicular dendritic cell neoplasia. Seven other lymph nodes were sent to high specialized reference centers because of diagnostic difficulties.
- 76 intracranial tumors were examined to differentiate between primary brain tumors, metastatic or meningeal tumors. The expression of GFAP was found in 42 tumors which were diagnosed as primary brain tumors (mainly astrocytoma or glioblastoma). Seven of the GFAP negative cases were diagnosed as meningeal tumors, 22 were diagnosed as metastatic carcinoma of various origin, 2 cases as metastatic melanoma, one case as B- cell lymphoma and 2 cases were interpreted as primary brain tumors and were sent to reference laboratories for further classification.
- 108 benign and malignant soft tissue tumors were examined to determine the histogenesis or to prove malignancy. 19 of these tumors examined were diagnosed as metastatic carcinoma or as tumor recurrence in the scar region. 32 lipomatous tumors were examined to exclude well differentiated liposarcoma based on the expression of MDM2 and the diagnosis of well differentiated liposarcoma was confirmed in 4 cases. 12 neurogenic tumors (neurofibroma) were

confirmed with neurogenic markers with low proliferation index. The diagnosis of Ewing's sarcoma / primitive neuroectodermal tumor was confirmed in 9 cases based on the expression of CD99 and absence of LCA; in 6 out of the 9 cases strong expression of FLI-1 was noted. Seven embryonal and 1 alveolar rhabdomyosarcoma were found positive for desmin and myogenin. Based on the morphology and characteristic immunoprofile the following soft tissue tumors were also diagnosed: 5 cases of granular cell tumor, 3 cases of biphasic synovial sarcoma, 2 cases of malignant nerve sheath tumor, 2 cases of kaposiform angiosarcoma, 2 cases of leiomyosarcoma, 1 case of alveolar soft part sarcoma, 1 case of malignant rhabdoid tumor, 1 case of clear cell sarcoma and 1 case of dermatofibrosarcoma protuberans. Two cases were classified as reactive inflammatory lesions and 9 were sent to international reference laboratories for further classification.

- 172 bone marrow trephines were examined for hematological malignancies, tumor staging or metastatic spread using tumor specific markers. 67 trephines were examined for lymphoma staging using B- and T- cell markers. 22 trephines were examined for myeloid neoplasia, 21 trephines were examined for plasma cell neoplasia, 61 trephines for carcinoma metastases of various origin and 3 trephines for neuroblastoma staging.
- Within the 19 examined suprarenal glands, the following tumors were confirmed by specific markers: metastatic spread of renal cell carcinoma in 9 cases, neuroblastoma in 2 cases, adrenal gland adenoma in 4 cases, pheochromocytoma in one case and hyperplastic changes in 3 cases.
- 191 breast carcinomas of different types were examined to determine the expression of estrogen- and progesterone receptors in addition to HER-2 for further tumor therapy. Five cases of endometrioid carcinomas and 2 cases of gastric adenocarcinomas were examined to determine the over-expression of HER-2. The expression of CD117 was also emphasized in 11 diagnosed gastrointestinal stromal tumors required for specific tumor therapy, while one additional tumor lacked the expression of CD117 but was positive for CD34 and Dog1.
- 384 cases received from outside were examined for a second opinion before tumor therapy. In 23 cases no adequate H&E slides or reliable immunostaining were available owing to suboptimal tissue fixation and / or bad tissue processing and additional, more suitable biopsies were recommended for certain diagnoses, which were later received. After careful examination of the H&E slides and optimal immunophenotyping, 347 out of the 384 outside diagnoses were

confirmed whereas 37 diagnoses were revised. Analyzing the causes of discrepancy between our and outside diagnoses we can divide the cases into the following categories:

- 19 out of the 37 revised diagnoses were made based on tumor morphology alone without doing any immunohistochemical study. It is noteworthy that 17 of these 19 diagnoses were differential diagnoses for tumors with similar morphology.
- In 8 cases, only a single immunohistochemical marker was used. The markers used were wide spectrum multilineage antibodies that lack the diagnostic specificity and include the following antibodies:
 - CD99 was positive in 2 cases diagnosed as Ewing's sarcoma; after complete immunophenotyping the diagnosis was corrected to acute lymphoblastic lymphoma.
 - S100 was used to prove malignant melanoma and the diagnosis was revised to metastatic malignant nerve sheath tumor.
 - TTF-1 was used to diagnose pulmonary adenocarcinoma and the diagnosis was revised to thyroid carcinoma.
 - CD138 positive cells diagnosed in a core biopsy as plasma cell neoplasia, after immunophenotyping the diagnosis was corrected to metastatic poorly differentiated squamous cell carcinoma.
 - EMA was used to prove the epithelioid origin of the neoplasia and the diagnosis was revised to anaplastic large cell lymphoma.
 - CD10 was used to support the diagnosis of metastatic renal cell carcinoma but the diagnosis was revised to hepatocellular carcinoma after further immunophenotyping.
 - Melan A to confirm metastatic melanoma and the diagnosis was revised to metastatic adrenocortical carcinoma.
- In 5 cases only 2 markers were used as listed below:
 - Two core biopsies with small cell tumors negative for CD3 and CD20; accordingly the lymphoma diagnosis was excluded by outside pathologists, after complete immunophenotyping both cases were finally diagnosed as acute lymphoblastic lymphoma.

- In one lymph node the tumor cells were positive for S100 but negative for pan-cytokeratin and the case was diagnosed as metastatic melanoma. After complete immunophenotyping the diagnosis was corrected to Langerhans cell sarcoma.
 - Metastatic tumor positive for CEA and pan-cytokeratin diagnosed as poorly differentiated adenocarcinoma, after considering the clinical data and the complete immunoprofile, the tumors was diagnosed as metastatic medullary thyroid carcinoma.
 - Retroperitoneal tumor positive for CD117 but negative for cytokeratin 7 diagnosed as epithelioid gastrointestinal stroma tumor; after complete immunophenotyping the diagnosis was corrected to metastatic seminoma.
 - In 5 cases there was misinterpretation of immunostaining results and the important differential diagnoses were neglected.
- 18 out of the 2691 tumors received remain unclear for us and were sent to highly specialized reference centers as consultations for a second opinion or for further molecular investigation.

7. Discussion

In modern diagnostic histopathology, immunohistochemistry plays a very important role as a very informative method for tumor diagnosis and management of oncologic patients. This method has been used since the 1940s and was primarily published by Coons et al.¹⁰¹ In the last 20 years, immunohistochemistry was dramatically developed into a highly specialized molecular technique combining the principles of immunology, biochemistry and histology and became a very powerful tool in the daily diagnostic histopathology. Immunohistochemistry is essential to determine the histogenetic origin of tumors required for tumor classification. It is also one of the efficient methods to detect minimal residual tumor cells in different locations such as surgical margins, lymph nodes and bone marrow, which is very important for tumor staging and the planning of therapeutic strategies. Immunohistochemistry is also helpful in determining the sensitivity of different tumors to several types of therapeutic agents such as steroid-receptor-antagonists, humanized monoclonal antibodies and enzyme antagonists. Furthermore, immunohistochemistry offers a number of significant prognostic and etiopathologic markers interesting for tumor follow-up and etiopathologic research. In this study - based on the adequate morphology and optimal immunophenotyping - we were able to characterize 2691 out of the 2709 examined cases (99.33% of the cases), while 198 cases were examined to determine the sensitivity of the tumors to therapeutic agents and 18 cases remain uncertain and were sent for a second opinion to highly specialized pathology centers. The results obtained emphasize the role of immunohistochemistry as a powerful tool in tumor histopathology. However, the experience showed that this powerful method is also very sensitive and any unprofessional use can be misleading, which frequently means negative therapeutic consequences for the patients. In the preceding sections we demonstrated that the majority of the available antibodies lacks the absolute specificity and a high professional level is required to construct rational and informative diagnostic panels and to interpret the results. Analyzing the diagnostic errors recorded in the past five years as consultants for immunohistochemistry using a wide spectrum of antibodies, we found that the errors listed below are the most common sources of error in diagnostic immunohistochemistry. It is noteworthy that these errors are occasionally linked together and need to be carefully analyzed so as to reach an optimal, safe and informative diagnostic level:

- Unprofessional supervision of the immunohistochemical laboratory and a lack of standardized approach are frequently found to be negative factors especially in new established laboratories. The optimal supervision and organization of the laboratory and the standardization of immunohistochemical techniques are foundation stones for successful immunohistochemical stain results. In our consultation work, we found few laboratories that have a high frequency of errors, obviously due to incompetent supervision and lack of standardization reflected by irregular or poor immunohistochemical stains with non-specific reaction or false negative and false positive results. Even the use of expensive automated immunohistochemical stain machines was not able to solve many of these problems. Attempts to accomplish better immunohistochemical stains in our laboratory on sections or blocks received from such laboratories were often unsuccessful as the antigenicity of the tissue was irreversibly lost due to inadequate tissue handling, improper fixation or bad tissue processing.
- The practice of the so called 'single marker immunohistochemistry' or the use of inappropriate panel of antibodies. This is a risky approach in diagnostic immunohistochemistry, frequently used by junior pathologists or because of financial constraints. Using one or two markers to solve a diagnostic problem can be the cause of serious diagnostic mistakes. In our practice we have recorded several misdiagnoses due to this approach, in which one or two broad-spectrum antibodies were used to confirm a hypothetical working diagnosis. To demonstrate the range and significance of mistakes caused by this approach, we mention below 3 repeatedly accruing examples. A frequent approach was the use of S100 protein to confirm the diagnosis of metastatic melanoma, after using a more specific panel; some of these tumors - previously diagnosed as metastatic melanoma - reveal a neuroendocrine or histiocytic origin. Another example which we noticed in a few cases is the use of CD99 as a single marker to confirm the diagnosis of Ewing's sarcoma in tumors with round cell morphology, but using a more specific antibody panel, including LCA and TdT, two of these diagnoses were corrected to acute lymphoblastic lymphoma. A further example was the use of epithelial membrane antigen (EMA) to prove the epithelial origin of the tumors; nevertheless two of these tumors diagnosed primarily as metastatic poorly differentiated carcinoma were corrected to anaplastic large cell lymphoma positive for CD30 and anaplastic lymphoma kinase (ALK). As demonstrated, the discrepancy between the diagnoses is very significant which, fundamentally changes the therapeutic strategies.

- Inadequate theoretical knowledge concerning the antibodies, including specificity and sensitivity, reactivity spectrum and cross reactivity; and yet such knowledge is essential for constructing sufficient and informative diagnostic antibody-panels. A wide range of polyclonal and monoclonal antibodies is now available, but each clone recognizes a specific domain within the antigen (see next point). Furthermore, different clones may have different cross reactivity with some other antigens, even with different stain pattern. As an example, we mention the MIB-1 clone of the Ki-67 antigen. Ki-67 reveals usually a nuclear stains pattern in the G₁, S, G₂ and M cell phases, but a membranous and cytoplasmic stain is reported using the MIB-1 clone in some tumors such as the hyalinizing trabecular adenoma / carcinoma of thyroid gland, pleomorphic adenoma of salivary glands and sclerosing hemangioma of the lung whereas this reaction phenomenon is not reported using other Ki-67 clones. Another interesting aspect is the possible difference of reactivity between monoclonal and polyclonal antibodies. An interesting example is the difference in reactivity of polyclonal and monoclonal CEA antibodies; polyclonal CEA antibody stains hepatocellular carcinoma and the majority of adenocarcinomas, whereas monoclonal CEA antibody is usually negative in hepatocellular carcinoma but positive in the majority of adenocarcinomas, a phenomenon important for differentiating between the two tumor identities.
- Inadequate theoretical knowledge regarding the biology of targeted antigens, including general structure, expression during different differentiation and cell cycle phases and expression pattern, stability and sensitivity to formalin fixation and tissue processing in addition to the most rational antigen retrieval method. It is always useful to have an idea about the structure of the antigens of interest and the targeted domain within the antigen molecule. It is important to keep in mind that different domains may have different locations in the cell structures and may have different stability to fixation and tissue processing, which may require different antigen unmasking methods. For correct interpretation, it is important to know the expression pattern of the targeted antigen (nuclear, cytoplasmic, membranous, extracellular or a combination of two sites), however some antigens have a different expression pattern, depending on the differentiation phase of the cell; an example is CD3 exhibiting a cytoplasmic expression in immature lymphoblastic stage but membranous stain in mature T- lymphocytes and related neoplasia. Another interesting example is the immunoprofile of the anaplastic large cell lymphoma, where the expression pattern of the anaplastic lymphoma kinase (nuclear or cytoplasmic) depends on the type of translocation

associated with this neoplasia.² Analyzing the cases reported as atypical expression pattern, we found that an unspecific background or error in the choice of the primary antibody was the most common cause mimicking atypical expression; nevertheless any atypical expression must be kept in mind and carefully analyzed.

The expression level and the stability of the antigens examined are also to be considered, some antigens must be carefully treated as they are very sensitive to formalin fixation and tissue processing, an example are steroid receptors and HER-2, whereas other antigens are very stable and can be easily detected even in necrotic tissue. Our experience showed that the ignorance of one or more of these factors can be a significant source of diagnostic mistakes.

- Occasional misinterpretation of stain results and the absence of standardized reporting schema are further important factors that may cause misdiagnosis. The precise interpretation of the immunohistological stains is very essential to reach the final accurate diagnosis. It is important to consider the expected stain pattern and to compare the stain of tumor structures with the available internal and external positive controls. Our experience showed that the ignorance of the internal control may lead to false positive or false negative results confusing the correct diagnosis. The negative control may also be of interest to exclude any unspecific stain or background.

8. Conclusions and recommendations

Immunohistochemistry is a powerful and sensitive diagnostic tool that requires high levels of practical and theoretical knowledge, which begins with the adequate processing of tissue samples and includes the standardized stain technique, optimal choice of diagnostic antibody panels and ends with critical interpretation of stain results. Based on our observations and the factors discussed above, we suggest in this section a list of different recommendations to utilize all the benefits of immunohistochemistry as an important method in diagnostic histopathology and to minimize possibilities of diagnostic errors:

1. The first important point to remember is that the careful histopathologic examination and clinical correlation remain the cornerstone of morphologic diagnosis. The immunostaining is usually able to support or rule out one or more of possible differential diagnoses.
2. The immunohistochemistry laboratory must be under the supervision of well trained pathologist who not only has the necessary morphologic knowledge, but who is highly skilled in methods and techniques of immunohistochemistry and is able to do good and critical interpretation of immunohistochemical results. We came to the conclusion that immunohistochemistry is safe and informative only in experienced, careful hands.
3. One of the most important lessons we learn from our experience is that single marker immunohistochemistry is one of the most frequent sources of error in tumor diagnosis with a high potential of serious diagnostic pitfalls in the differential diagnosis, especially between malignant tumors with similar morphology. No single marker can be relied on exclusively and the use of adequate panel of antibodies helps to avoid misinterpretation; it is always advisable to confirm or exclude the diagnosis by two or more additional antibodies.
4. Knowledge of the nature of targeted antigens is a very important factor in the interpretation of the results. The following detail must be always considered:
 - The expression pattern of the antigen (nuclear, cytoplasmic, membranous or extracellular).

- Stability of antigens during tissue processing. Optimal and standardized tissue fixation and processing is essential for good immunostaining. It is always important to remember that bad H&E sections mean bad immunohistochemistry results.
 - It is always important to consider that histopathologists are dealing with neoplasia composed of a heterogeneous cell population with high potential of genotypic and phenotypic variations and any atypical expression of antigens must be documented and analyzed. The reason for the atypical expression can be the biology of the tumor or the nature of the antibodies used, as some antibodies or clones can demonstrate cross-reaction between different cell or tissue structures.
5. Knowledge of the nature of the antibodies used: The details of any new antibody used must be carefully studied. The following parameters must be always considered:
- Type of the antibody: polyclonal or monoclonal (mouse or rabbit monoclonal antibody).
 - Clone of monoclonal antibody.
 - Sensitivity, specificity of the antibody in addition to stain pattern.
 - The recommended dilution of concentrated antibodies, whereas the final dilution must be determined in the laboratory according to the technique used, incubation time and detection method used in the laboratory.
 - Care must be exercised when using newly developed antibodies. New antibodies are often introduced as being highly specific, but after prolonged use or testing on tissue microarrays many of them prove to be less specific.
6. The specificity and sensitivity of the detection system are important factors for optimal staining, which can affect the interpretation. Currently, different one-step and two-step detection systems are available from different companies. Any new system must be examined and optimized in the laboratory and the results must be compared with other available systems.
7. The standardization of the immunohistochemical method is one of the essential factors for correct interpretation of stain results. We must be sure that the stain results are always reproducible. Factors that must be standardized include tissue fixation and tissue processing, clone of the antibodies and dilution, antigen retrieval method and detection system, incubation time and incubation temperature, washing steps and chromogen. The modification of any of the factors

mentioned can cause variations of stain results and any changes in the stain technique must be tested and standardized before being accepted as a part of the routine staining technique.

8. Positive and negative controls are valuable for good interpretation. The permanent use of positive controls is recommended for many antibodies, especially for new and highly sensitive ones; however the best positive and negative controls are the internal controls which may be present in the examined tissue. In any case, care should always be exercised when comparing reaction results of ideally collected and processed positive tissue control with unknown tissue samples containing tumor with heterogeneous cell population. In our laboratory we use positive and negative controls only for new antibodies, sensitive antigens or in the case of small and valuable tissue samples. Occasionally it is important to examine the antigenicity of the tissue, for this purpose a vimentin or CD34 stain is sufficient.
9. The interpretation and documentation of immunohistochemical results must be standardized. It is not enough to interpret the staining result as positive or negative. Quality of stain and staining pattern, quantity and intensity of stained structures must be also considered and documented and any conflicting results must be analyzed. Standardized reporting is very helpful in organizing the information to reach an accurate diagnosis.
10. Despite the high sensitivity of immunohistochemistry and the large number of available antibodies, immunohistochemistry - as any method - has its own limits. We should never force the diagnosis based on unclear or unspecific results. Some cases must be clarified or confirmed by additional methods. The detection of translocations or other genetic abnormalities associated with various types of neoplasia by molecular methods is an example where we need other methods supplement immunohistochemistry in order to obtain a more precise tumor diagnosis.

Taking into consideration the above mentioned factors will positively increase the value of immunohistochemistry in histopathologic diagnosis and minimize the frequency of diagnostic errors.

9. Summary

Immunohistochemistry is one of the methods that enables the detection and visualization of different antigens in cells and tissue sections using specific primary antibodies and an efficient detection system. This method has been used since the 1940s and was primarily published by Coons et al.¹⁰¹ In the last 20 years, immunohistochemistry was dramatically developed into a highly specialized molecular technique combining the principles of immunology, biochemistry and histology and became a very powerful tool in the daily diagnostic histopathology. A large number of diagnostic antibodies are now available to detect and localize a large number of cell and tissue antigens that enable histopathologists to resolve many diagnostic problems and to increase diagnostic certainty. Nevertheless, immunohistochemistry - as any other method - has its own possibilities, limitations and diagnostic pitfalls that are important to know for everyone practicing this method.

Based on our experience as a reference laboratory for immunohistochemistry, we initiate this study to analyze the possibilities, limitations and pitfalls of immunohistochemistry as an important diagnostic tool in the daily practice of modern histopathologists. In this work we describe the standardized immunohistochemical technique used in our practice and demonstrate our diagnostic algorithms developed to be used as a guide for the classification of primary tumors and histogenetic identification of metastases of unknown origin. We also describe the most important antibodies used in the daily routine and the rational use of these antibodies with the most informative, labor- and time-saving combinations. We discuss the distribution and the specific expression pattern of the targeted antigens and point out the most characteristic immunoprofiles for a large number of tumors and the diagnostic pitfalls that must be considered to avoid any misinterpretation.

Considering the factors mentioned above, we analyze in this study the results of 18000 immunohistochemical stains performed in the past 5 years to study 2907 various tumors using more than 110 specific monoclonal and polyclonal primary antibodies. Depending on the adequate tumor morphology and optimal immunophenotyping, we were able to characterize 2691 out of the 2709 cases examined, while 18 cases remain uncertain and were sent for a second opinion to highly specialized centers. Further 198 cases were only tested to determine the sensitivity of the tumors to specific therapeutic agents. In the same time we were able to revise 37 outside diagnoses, mainly

made without or only with limited immunohistochemical study. After the analysis of error sources, we present our conclusions and recommendations to increase the value of immunohistochemistry as an informative diagnostic method.

The results obtained confirmed the role of immunohistochemistry as a powerful tool in tumor histopathology; however the experience showed that this powerful method is safe and informative only in experienced and careful hands.

10. References

1. Sh-R Shi, Ch Liu, C R Taylor. Standardization of immunohistochemistry for formalin-fixed, paraffin-embedded tissue sections based on the antigen-retrieval technique: from experiments to hypothesis. *J Histochem Cytochem* 20007; 55:105-109.
2. M Tuffaha. Phenotypic and genotypic diagnosis of malignancies. Immunohistochemical and molecular approach in tumor diagnosis and detection of minimal residual cancer disease. Wiley-VCH- Verlag, Weinheim, Berlin 2008.
3. A Bahrami, L D Truong, J Y. Ro. Undifferentiated tumor true identity by immunohistochemistry. *Arch Pathol Lab Med* 2008; 132: 326-348.
4. R Moll. Initiale CUP-Situation und CUP-Syndrom. *Pathologe* 2009; 30: 1-7.
5. Iwata, Fletcher. Immunohistochemical detection of cytokeratin and epithelial membrane antigen in leiomyosarcoma: a systemic study of 100 cases. *Pathol Int* 2000; 50: 7-14.
6. R Moll, M Divo, L Langbein. The human keratins: biology and pathology. *Histochem Cell Biol* 2008; 129: 705-733.
7. P G Chu, S K Lau, L M Weiss. Keratin Expression in Endocrine Organs and their Neoplasms. *Endocr Pathol* 2009; 20: 1-10.
8. J Plaza, D Perez-Montiel, J Mayerson, et al. Metastases to soft tissue. A review of 118 cases over a 30-year period. *Cancer* 2008; 112: 193-203.
9. P Chu, E Wu, L M Weiss. Cytokeratin 7 and cytokeratin 20 expression in epithelial neoplasms: A survey of 435 cases. *Mod Pathol* 2000; 13: 962-972.
10. X Lin, R S. Saad, T M. Luckasevic, et al, Diagnostic value of CDX-2 and TTF-1 expressions in separating metastatic neuroendocrine neoplasms of unknown origin. *Appl Immunohistochem Mol Morphol* 2007; 15: 407-414.

11. E Comp rat, F Zhang, C Perrotin, et al. Variable sensitivity and specificity of TTF-1 antibodies in lung metastatic adenocarcinoma of colorectal origin. *Mod Pathol* 2005; 18: 1371-1376.
12. R W Werling, H Yaziji, C E Bacchi, A M Gown. CDX2, a highly sensitive and specific marker of adenocarcinomas of intestinal origin: an immunohistochemical survey of 476 primary and metastatic carcinomas. *Am J Surg Pathol*. 2003; 27: 303-10.
13. R M Mazziotta, A C Borczuk, C A Powell, et al. CDX2 immunostaining as a gastrointestinal marker: expression in lung carcinomas is a potential pitfall. *Appl Immunohistochem Mol Morphol*; 2005; 13: 55-60.
14. P H Levine, A Joutovsky, J Cangiarella, et al. CDX-2 expression in pulmonary fine-needle aspiration specimens: a useful adjunct for the diagnosis of metastatic colorectal adenocarcinoma. *Diagn Cytopathol* 2006; 34: 191-195.
15. F Miselli, C Millefanti, E Conca, et al. PDGFRA immunostaining can help in the diagnosis of gastrointestinal stromal tumors. *Am J Surg Pathol* 2008; 32:738-743.
16. I Espinosa, C-H Lee, M K Kim, et al. A novel monoclonal antibody against DOG1 is a sensitive and specific marker for gastrointestinal stromal tumors. *Am J Surg Pathol* 2008; 32:210-218.
17. M Miettinen, Z-F Wang, W, J Lasot. DOG1 Antibody in the differential diagnosis of gastrointestinal stromal tumors. A study of 1840 cases. *Am J Surg Pathol* 2009; 33: 1401-1408.
18. B Liegl, J L Hornick, Ch L Corless, et al. Monoclonal Antibody DOG1.1 Shows Higher Sensitivity than KIT in the Diagnosis of Gastrointestinal Stromal Tumors, Including Unusual Subtypes. *Am J Surg Pathol* 2009; 33:437-446.
19. L W Lamps, A L Folpe, The diagnostic value of hepatocyte paraffin antibody 1 in differentiating hepatocellular neoplasms from nonhepatic tumors: a review. *Adv Anat Pathol* 2003; 10: 39-43.

20. P G Chu, Sh Ishizawa, E Wu, L M Weiss. Hepatocyte antigen as a marker of hepatocellular carcinoma: An immunohistochemical comparison to carcinoembryonic antigen, CD10, and alpha-fetoprotein. *Am J Surg pathol* 2002; 26: 978-988.
21. Ch-Ch Pan, P Ch-H Chen, Sh-H Tsay, D M-T Ho. Differential immunoprofiles of hepatocellular carcinoma, renal cell carcinoma, and adrenocortical carcinoma. A systemic immunohistochemical survey using tissue array technique. *Appl Immunohistochem Mol Morphol* 2005; 13:347-352.
22. Z Fan, M van de Rijn, K Montgomery, R V Rouse. Hep Par 1 antibody stain for the differential diagnosis of hepatocellular carcinoma: 676 tumors tested using tissue microarrays and conventional tissue sections. *Mod Pathol* 2003;16(2):137-144.
23. M A Watson, S Dintzis, Ch M. Darrow, et al. Mammaglobin expression in primary, metastatic and occult breast cancer. *Cancer research* 1999; 59, 3028-3031.
24. E Sasaki, N Tsunoda, Y Hatanaka, et al. Breast-specific expression of MGB1/ mammaglobin: an examination of 480 tumors from various organs and clinicopathological analysis of MGB1-positive breast cancers. *Mod Pathol* 2007; 20: 208-214.
25. P Viacava, A G Naccarato, G Bevilacqua. Spectrum of GCDFP-15 expression in human fetal and adult normal tissues. *Virchows Archive*, 1998; 432: 255-60.
26. Mc Gregor, K David, K Khurana, et al. Diagnosing primary and metastatic renal cell carcinoma. *Am J Surg pathol* 2001; 25: 1485-1492.
27. H-Y Wang, St E Mills. KIT and RCC are useful in distinguishing chromophobe renal cell carcinoma from the granular variant of clear cell renal cell carcinoma. *Am J Surg Pathol* 2005; 29: 640-646.
28. A K Avery, J Beckstead, A A Renshaw, et al. Use of antibodies to RCC and CD10 in the differential diagnosis of renal neoplasms. *Am J Surg Pathol* 2000; 24: 203-210.

29. D Cao, A Maitra, J-A Saavedra, et al. Expression of novel markers of pancreatic ductal adenocarcinoma in pancreatic nonductal neoplasms: additional evidence of different genetic pathways. *Mod Pathol* 2005; 18, 752-761.
30. X-R Wu, J-H Lin, Th Walzg, et al. Mammalian uroplakins: A group of highly conserved urothelial differentiation-related membrane proteins. *J of biological biochemistry* 1994; 269: 13716-13724.
31. E D Lobban, B A Smith, G D Hall, et al. Uroplakin gene expression by normal and neoplastic human urothelium. *Am J of Pathol* 1998; 153: 1957-1967, 1998.
32. T Yuasa, T Yoshiki, T Isono, et al. Expression of transitional cell-specific genes, uroplakin Ia and II, in bladder cancer: detection of circulating cancer cells in the peripheral blood of metastatic patients. *Int J Urol.* 1999; 6: 286-292.
33. O Kaufmann, J Volmerig, M Dietel. Uroplakin III is a highly specific and moderately sensitive immunohistochemical marker for primary and metastatic urothelial carcinomas. *Am J Clin Pathol* 2000; 113: 683-687.
34. Z Jiang, B A Woda, C L Wu, X J Yang. Discovery and clinical application of a novel prostate cancer marker: alpha methylacyl CoA racemase (P504S). *Am J Clin Pathol* 2004; 122: 275-89.
35. Z Jiang, K A Iczkowski, B A Woda, et al. P504S immunostaining boosts diagnostic resolution of “suspicious” foci in prostatic needle biopsy specimens. *Am J Clin Pathol* 2004; 121: 99-107.
36. G Paner, D J Luthringer, M B Amin. Best practice in diagnostic immunohistochemistry prostate carcinoma and its mimics in needle core biopsies. *Arch Pathol Lab Med* 2008; 132:1388-1396.
37. B F Skinner, E Oliva, R H. Young, et al. Expression of -methylacyl-CoA racemase (P504S) in nephrogenic adenoma. A significant immunohistochemical pitfall compounding the differential diagnosis with prostatic adenocarcinoma. *Am J Surg Pathol* 2004; 28:701-705.

38. K Biermann, D Klingmueller, A Koch, et al. Diagnostic value of markers M2A, OCT3/4, AP-2c, PLAP and c-KIT in the detection of extragonadal seminomas. *Histopathol* 2006; 49: 290-297.
39. A Bahrami, J Y Ro, A G Ayala. An overview of testicular germ cell tumors. *Arch Pathol Lab Med* 2007; 131: 1267-1280.
40. Th M Ulbright. Germ cell tumors of the gonads: a selective review emphasizing problems in differential diagnosis, newly appreciated, and controversial issues. *Mod Pathol* 2005; 18: 61-79.
41. T D Jones, Th M Ulbright, J N Eble, et al. OCT4: a sensitive and specific biomarker for intratubular germ cell neoplasia of the testis. *Clinical Cancer Research* 2004; 10: 8544-8547.
42. L H J Looijenga, H Stoop, H P J C de Leeuw, et al. POU5F1 (OCT3/4) identifies cells with pluripotent potential in human germ cell tumors. *Cancer Research* 2003; 63: 2244-2250.
43. Th M Ulbright, The most common, clinically significant misdiagnoses in testicular tumor pathology, and how to avoid them. *Adv Anat Pathol* 2008; 15:18-27.
44. S K Lau, L M Weiss, P G Chu. D2-40 immunohistochemistry in the differential diagnosis of seminoma and embryonal carcinoma: a comparative immunohistochemical study with KIT (CD117) and CD30. *Mod Pathol* 2007; 20: 320-325.
45. G Fraternali-Orcioni, B Falini, F Quaini, et al. Beta-HCG aberrant expression in primary mediastinal large B-cell lymphoma. *Am J Surg Pathol* 1999; 23:717-21.
46. Robert H Young. Sex cord-stromal tumors of the ovary and testis: their similarities and differences with consideration of selected problems. *Modern Pathology* 2005; 18, 81-98.
47. K Kontogianni, A G Nicholson, D Butcher, M N Sheppard. CD56: a useful tool for the diagnosis of small cell lung carcinomas on biopsies with extensive crush artifact. *J Clin Pathol* 2005; 58:978-980.

48. D S Klimstra, I R Modlin, V Adsay, et al. Pathology Reporting of Neuroendocrine Tumors: Application of the Delphic Consensus Process to the Development of a Minimum Pathology Data Set. *Am J Surg Pathol* 2010; 34:300-313.
49. S Fischer, S L Asa. Application of immunohistochemistry to thyroid neoplasms. *Arch Pathol Lab Med.* 2008; 132:359-372.
50. H Sasano, T Suzuki, T Moriya. Recent advances in histopathology and immunohistochemistry of adrenocortical carcinoma. *Endocr. Pathol.* 2006; 17:345-5-354.
51. M Miettinen, J Chatten, A Paetau. Monoclonal antibody NB84 in the differential diagnosis of neuroblastoma and other small round cell tumors. *Am J Surg pathol* 1998; 22. 327-32.
52. A de C Carvalho, E R Parra, M C Zerbini, et al. Morphometric evaluation on NB84, synaptophysin and AGNOR is useful for the histological diagnosis and prognosis in peripheral neuroblastic tumors (PNTS). *Clinics* 2007; 62:731-40.
53. A N Husain, Th V Colby, N G Ordoñez, et al. Guidelines for pathologic diagnosis of malignant mesothelioma. A consensus statement from the international mesothelioma Interest Group. *Arch Pathol Lab Med.* 2009; 133: 1317-1331.
54. A M Marchevsky. Application of Immunohistochemistry to the Diagnosis of Malignant Mesothelioma. *Arch Pathol Lab Med.* 2008; 132:397-401.
55. J E King, N Thatcher, C A C Pickering, P S Hasleton. Sensitivity and specificity of immunohistochemical markers used in the diagnosis of epithelioid mesothelioma: a detailed systematic analysis using published data. *Histopathol* 2006; 48: 223-32.
56. A Y Chu, L A Litzky, Th L Pasha, et al. Utility of D2-40, a novel mesothelial marker, in the diagnosis of malignant mesothelioma. *Mod Pathol* 2005; 18: 105-110.

57. L Browning, D Bailey, A Parker. D2-40 is a sensitive and specific marker in differentiating primary adrenal cortical tumours from both metastatic clear cell renal cell carcinoma and pheochromocytoma. *J Clin Pathol* 2008; 61: 293-296.
58. R A Higgins, E Blankenship, M C Kinney. Application of immunohistochemistry in the diagnosis of non-Hodgkin and Hodgkin lymphoma. *Arch Pathol Lab Med* 2008; 132:441-461.
59. X F Zhao. Pitfalls in diagnostic hematopathology: Part I. *Int J Clin Exp Pathol* 2009; 2, 11-20.
60. X F Zhao. Pitfalls in diagnostic hematopathology: Part II. *Int J Clin Exp Pathol* 2010; 3:39-46.
61. C J Buresh, B R Oliai, R T Miller. Reactivity with TdT in Merkel cell carcinoma. A potential diagnostic pitfall. *Am J Clin Pathol* 2008; 129: 894-898.
62. M Sur, H AlArdati, C Ross, et al. TdT expression in Merkel cell carcinoma: potential diagnostic pitfall with blastic hematological malignancies and expanded immunohistochemical analysis. *Mod Pathol* 2007; 20: 1113-1120.
63. J Ordi, C Romagosa, F A Tavassoli, et al. CD10 expression in epithelial tissues and tumors of the gynecologic tract. A useful marker in the diagnosis of mesonephric, trophoblastic, and clear cell tumors. *Am J Surg pathol* 2003; 2: 178-186.
64. N Borscheri, A Roessner, Ch Röcken. Canalicular immunostaining of neprilysin (CD10) as a diagnostic marker for hepatocellular carcinomas. *Am J Surg Pathol* 2001; 25: 1297-1303.
65. N Masir, T Marafioti, M Jones, et al. Loss of CD19 expression in B-cell neoplasms. *Histopathol* 2006; 48:239-46.
66. K C Jensen, J PT Higgins, K Montgomery, et al. The utility of PAX5 immunohistochemistry in the diagnosis of undifferentiated malignant neoplasms. *Mod Pathol* 2007; 20: 871-877.
67. A L Feldman, A Dogan. Diagnostic uses of Pax5 immunohistochemistry. *Adv Anat Pathol* 2007; 14:323-334.

68. L M Sullivan, K A Atkins, R D LeGallo. PAX immunoreactivity identifies alveolar rhabdomyosarcoma. *Am J Surg Pathol* 2009; 33:775-780.
69. D A Morgenstern, S Gibson, N J Sebire, J Anderson. PAX5 expression in rhabdomyosarcoma. *Am J Surg Pathol* 2009; 33: 1575-1577.
70. P G Chu, D A Arber, L M Weiss. Expression of T/NK-cell and plasma cell antigens in non-hematopoietic epithelioid neoplasms. An immunohistochemical study of 447 cases. *Am J Clin Pathol* 2003; 120:64-70.
71. E Matutes. New additions to antibody panels in the characterization of chronic lymphoproliferative disorders *J Clin Pathol* 2002; 55:180-183.
72. A Islam, A O Vladutiu, Th Donahue, et al. CD8 Expression on B Cells in Chronic Lymphocytic Leukemia. A Case Report and Review of the Literature. *Arch Pathol Lab Med.* 2000; 124:1361-1363.
73. S A Pileri, S Ascani, L Leoncini, et al. Hodgkin's lymphoma: the pathologist's viewpoint. *J Clin Pathol* 2002; 55: 162-176.
74. M G Bayerl, G Bentley, M C Bellan, et al. Lacunar and Reed-Sternberg-like cells in follicular lymphomas are clonally related to the centrocytic and centroblastic cells as demonstrated by laser capture microdissection. *Am J Clin Pathol* 2004; 122: 858-864.
75. J H Shanks, S S Banerjee. VS38 immunostaining in melanocytic lesions. *J Clin Pathol* 1996; 49: 205-207.
76. Y Natkunam, R A Warnke, K Montgomery, et al. Analysis of MUM1/IRF4 protein expression using tissue microarrays and immunohistochemistry *Mod Pathol* 2001;14:686-694.
77. JH Jordan, S Walchshofer, W Jurecka, et al. Immunohistochemical properties of bone marrow mast cells in systemic mastocytosis: Evidence for expression of CD2, CD117/Kit, and bcl-xL. *Hum Pathol* 2001; 32:545-52.

78. P L A van Daele, B S Beukenkamp, W M C Geertsma-Kleinekoort, et al. Immunophenotyping of mast cells: a sensitive and specific diagnostic tool for systemic mastocytosis. *Neth J Med* 2009; 67; 142-6.
79. D A Biddle, J Y Ro, G S Yoon, et al. Extranodal follicular dendritic cell sarcoma of the head and neck region: Three new cases, with a review of the literature. *Mod Pathol* 2002; 15:50-58.
80. E M Gaertner, M Tsokos, G A Derringer, et al. Interdigitating dendritic cell sarcoma: A report of four cases and review of the literature *Am J Clin Pathol* 2001; 115:589-597.
81. S Kairouz, J Hashash, W Kabbara, et al. Dendritic cell neoplasms: An overview. *Am. J. Hematol.* 2007; 82: 924-928.
82. Q Xie, L Chen, K Fu, et al. Podoplanin (D2-40): A new immunohistochemical marker for reactive follicular dendritic cells and follicular dendritic cell sarcomas. *Int J Clin Exp Pathol* 2008; 1: 276-284.
83. B Perry, C Cohen, B Govindarajan, et al. Wilms Tumor 1 Expression Present in Most Melanomas but Nearly Absent in Nevi. *Arch Dermatol.*2006; 142: 1031-1034.
84. R A Higgins, J E Blankenship, M C Kinney. Application of Immunohistochemistry in the diagnosis of non-Hodgkin and Hodgkin lymphoma. *Arch Pathol Lab Med.* 2008;132: 441-461.
85. W Al-Daraji, E Husain, B G Zelger, et al. A Practical and Comprehensive Immunohistochemical approach to the diagnosis of superficial soft tissue tumors. *Int J Clin Exp Pathol* 2009; 2: 119-131.
86. J Heim-Hall, L Yohe. Application of immunohistochemistry to soft tissue neoplasms. *Arch Pathol Lab Med.* 2008; 132: 476-489.
87. M H Cessna, H Zhou, SL Perkins, et al. Are myogenin and MyoD1 expression specific for rhabdomyosarcoma?: A study of 150 cases, with emphasis on spindle cell mimics. *Am J Surg Pathol* 2001; 25:1150-7.

88. A Heerema-McKenney, L C D Wijnaendts, J F Pulliam, et al. Diffuse myogenin expression by immunohistochemistry is an independent marker of poor survival in pediatric rhabdomyosarcoma. A tissue microarray study of 71 primary tumors including correlation with molecular phenotype. *Am J Surg Pathol* 2008; 32:1513-1522.
89. R A Morotti, K K Nicol, D M Parham, et al. An immunohistochemical algorithm to facilitate diagnosis and subtyping of rhabdomyosarcoma: The children's oncology group experience. *Am J Surg Pathol* 2006; 30: 962-968.
90. Ch-H Lee, D A Turbin, Y-C V Sung, et al. A panel of antibodies to determine site of origin and malignancy in smooth muscle tumors. *Mod Pathol* 2009; 22: 1519-1531.
91. J D Goldsmith, B Pawel, J R. Goldblum, et al. Detection and diagnostic utilization of placental alkaline phosphatase in muscular tissue and tumors with myogenic differentiation. *Am J Surg Pathol* 2002; 26: 1627-1633.
92. M P Pusztaszeri, W Seelentag, F T. Bosman. Immunohistochemical expression of endothelial markers CD31, CD34, von Willebrand factor, and Fli-1 in normal human tissues. *J Histochem Cytochem* 2006; 54:385-395.
93. S Breiteneder-Geleff, A Soleiman, H Kowalski, et al. Angiosarcomas express mixed endothelial phenotypes of blood and lymphatic capillaries: podoplanin as a specific marker for lymphatic endothelium. *Am J Pathol* 1999; 154: 385-394.
94. J-M Coindre, F Pédeutour, A Aurias. Well-differentiated and dedifferentiated liposarcomas. *Virchows Arch* 2010; 456:167-79.
95. M B N Binh, X Sastre-Garau, L Guillou, et al. MDM2 and CDK4 immunostainings are useful adjuncts in diagnosing well-differentiated and dedifferentiated liposarcoma subtypes. A Comparative analysis of 559 soft tissue neoplasms with genetic data. *Am J Surg Pathol* 2005; 29: 1340-1347.

96. M B N Binh, X S Garau, L Guillou, et al. Reproducibility of MDM2 and CDK4 staining in soft tissue tumors. *Am J Clin Pathol* 2006; 125:693-697.
97. L K Campbell, J R Thomas, L W Lamps, et al. Protein gene product 9.5 (PGP 9.5) is not a specific marker of neural and nerve sheath tumors: An immunohistochemical study of 95 mesenchymal neoplasms. *Mod Pathol* 2003; 16:963-969.
98. A L. Folpe, Ch E. Hill, D M Parham, et al. Immunohistochemical detection of FLI-1 protein expression. A study of 132 round cell tumors with emphasis on CD99-positive mimics of Ewing's sarcoma / primitive neuroectodermal tumor. *Am J Surg pathol* 2000; 24: 1657-1662.
99. S Rossi, E Orvieto, A Furlanetto, et al. Utility of the immunohistochemical detection of FLI-1 expression in round cell and vascular neoplasm using a monoclonal antibody. *Mod Pathol* 2004; 17: 547-552.
100. O Kaufmann, E Fietze, J Mengers, and M Dietel. Value of p63 and cytokeratin 5/6 as immunohistochemical markers for the differential diagnosis of poorly differentiated and undifferentiated carcinomas. *Am J Clin Pathol* 2001; 116:823-830.
101. A H Coons, H J Creech and R N Jones. Immunological properties of an antibody containing a fluorescent group. *Proc Soc Exp Biol Med* 1941; 47:200-202.

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Curriculum vitae

**"Mein Lebenslauf wird aus datenschutzrechtlichen Gründen in der elektronischen
Version meiner Arbeit nicht veröffentlicht."**

Publications

Phenotypic and genotypic diagnosis of malignancies. Immunohistochemical and molecular approach in tumor diagnosis and detection of minimal residual cancer disease.

Al Bodour publisher, Damascus, Syria, April 2002. First edition (ISBN: 9957-8545-0-X).

Phenotypic and genotypic diagnosis of malignancies. Immunohistochemical and molecular approach in tumor diagnosis and detection of minimal residual cancer disease.

The new printing company, Amman, Jordan, April 2005. Second edition (ISBN: 9957-8545-1-8).

Phenotypic and genotypic diagnosis of malignancies. An immunohistochemical and molecular approach

Wiley-VCH-Verlag, Weinheim, Berlin, March 2008. Third edition (ISBN 3-527-31881-X).

Eidesstattliche Erklärung

„Ich, Muin Tuffaha, erkläre, dass ich die vorgelegte Dissertation mit dem Thema „Immunohistochemistry in diagnostic pathology of tumors. Approach, benefits, limits and pitfalls“ selbst und ohne die unzulässige Hilfe Dritter verfasst, keine anderen als die angegebenen Quellen und Hilfsmittel benutzt und auch in Teilen keine Kopien anderer Arbeiten verwendet habe.

Berlin, den 28. Juni 2010

Muin Tuffaha