THE PROGNOSTIC ROLE OF IMMUNOHISTOCHEMISTRY IN SARCOMAS

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<table>
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<th>• IHC plays central role in sarcoma diagnosis</th>
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<tr>
<td>– Confirm histologic impression</td>
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<td>– Distinguish among histologically similar tumors</td>
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<td>– Support diagnosis of rare tumor type</td>
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<td>– Support diagnosis when tumor arises in unusual location or unusual age group</td>
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IMMUNOHISTOCHEMISTRY IN SARCOMA CLASSIFICATION

• Diagnostic IHC markers for sarcomas:
  – Markers of differentiation/lineage
    » SMA, desmin, caldesmon, myogenin, S-100 protein, KIT, EMA, keratins
  – Protein correlates of molecular findings
    » ALK, MDM2/CDK4, TFE3, INI1, TLE1
Inflammatory WDLPS

MDM2
Alveolar Soft Part Sarcoma

TFE3
TRADITIONAL PROGNOSTIC MARKERS FOR CANCER

- Tumor size
- Stage
- Lymph node metastases
- Grade
- Vascular invasion
REQUIREMENTS OF NEW PROGNOSTIC MARKER FOR ACCEPTANCE IN CLINICAL PRACTICE

• Assessment is reproducible and widely available with quality controls

• Substantial added value beyond established prognostic markers

• Results confirmed by additional independent studies

TYPES OF PROGNOSTIC MARKER STUDIES IN CANCER

• Phase I: Exploratory studies (hypothesis generating): seek association between potential marker and disease characteristics that have prognostic importance (grade, stage)

• Phase II: Exploratory studies: evaluate ability of prognostic marker to discriminate between patients at high and low risk of disease progression or death

• Phase III: Confirmatory studies (preferably large, collaborative); independent data sets

PROBLEMS WITH PROGNOSTIC MARKER STUDIES

• How to determine sample size?
• Cutpoint used to dichotomize continuous marker into “high” (positive) or “low” (negative)?
• Multivariable regression including all known prognostic markers/factors (independent significance)
• Publication bias (selective reporting)
• Lack of systematic reviews and/or meta-analyses
PROBLEMS WITH PROGNOSTIC MARKER STUDIES FOR SARCOMA

• Rare diseases – small sample sizes
• Variability in diagnosis/classification among pathologists
• Variability in treatment among oncologists
• Prospective studies nearly impossible
IMMUNOHISTOCHEMISTRY IN SARCOMA PROGNOSTICATION?

• No currently accepted clinical role, beyond classification/diagnosis (along with grade, most important prognostic factors)

• Numerous “biomarkers” evaluated, most in single retrospective studies

• “Traditional” markers (>20 yrs) such as Ki-67 and p53

• Newer markers identified through gene expression profiling
Ki-67 (MIB-1)

- Recognizes nuclear antigen
- Expressed in proliferating cells
- Preferentially late G1, S, G2, M
- Not expressed in G0
- Widely used in pathology as marker of proliferation; criterion for malignancy or grade (some organ systems)
p53

- Key role in cell cycle and apoptosis
- Tumor suppressor gene commonly mutated in diverse cancers
- Wild-type protein weak/negative by IHC
- Mutant protein extended half-life, detectable by IHC (“overexpression”)
- Strong positive staining by IHC reasonable correlation with p53 mutation
1. Prognostic studies including all types of soft tissue sarcomas
2. Myogenic differentiation in pleomorphic sarcomas
3. Extent of myogenin expression in rhabdomyosarcoma
4. Tumor type-specific prognostic studies
PROGNOSTIC STUDIES INCLUDING ALL TYPES OF SOFT TISSUE SARCOMAS
174 adult soft tissue sarcomas (heterogeneous types)
IHC for p53 and Ki-67
>20% cut-off for each
• Decreased survival for p53+ and high Ki-67

• No significant difference among high grade sarcomas

• Neither marker prognostic significance in multivariate analysis
Levine et al.

- 52 adult soft tissue sarcomas (heterogeneous types)
- IHC for Ki-67
- >40% cut-off = “high”
  - 84% “low” Ki-67
  - 16% “high” Ki-67
• Decreased survival for high Ki-67

• Grade not included in multivariate analysis

- 216 soft tissue sarcomas (heterogeneous types)
- IHC for Ki-67 and p53
- >10% cut-off for p53
- >12% cut-off for Ki-67 (median)

- Grade strongest predictor of survival
- No effect of Ki-67 on survival for “MFH”
- Among high grade sarcomas other than “MFH”, Ki-67 strong independent predictor of survival

- 86 primary extremity soft tissue sarcomas (heterogeneous types)
- IHC for p53 and MDM2
- >10% cut-off for each
  - 57% positive for p53
  - 70% positive for MDM2
  - 46% positive for both
121 localized high grade soft tissue sarcomas of extremities (heterogeneous types)

IHC for Ki-67 and p53

>20% cut-off for each

- Ki-67 “increased” in 69%
- p53 “positive” in 9%
Metastasis-free survival

Not significant

• Ki-67 independent prognostic significance for both metastasis and overall survival

Significant
Comments

- Ki-67 not a replacement for grading
- Ki-67 may be prognostic for (some) high grade sarcomas
- p53 aberrations nearly exclusive to high grade sarcomas
- Given marked differences in behavior for sarcoma subtypes, difficult to draw meaningful conclusions
MYOGENIC DIFFERENTIATION IN PLEOMORPHIC SARCOMAS
Fletcher et al.

- 100 “MFH” of extremities/trunk wall re-classified
- Upon re-review:
  - 29 myxofibrosarcomas
  - 20 leiomyosarcomas
  - 30 overall some form of high grade myogenic sarcoma
Myogenic tumors (stage II/III) worse metastasis-free survival

- 92 pleomorphic sarcomas of extremities
- IHC for SMA, MSA, desmin, myoglobin
  - 42 positive for at least 1 marker
Myoid differentiation independent adverse prognostic indicator
Inverse relationship between number of positive myoid markers and survival
• 65 pleomorphic sarcomas of extremities re-evaluated

• Upon re-review:
  – 22 leiomyosarcomas
  – 13 myxofibrosarcomas
  – 9 other myogenic sarcomas
Myogenic differentiation only independent predictor of overall survival
Comments

- Subclassification of pleomorphic sarcomas clinically significant
- IHC plays important role identify/confirm myogenic sarcomas
- Pleomorphic sarcomas with myogenic differentiation (not only LMS, RMS) higher metastatic potential
EXTENT OF MYOGENIN EXPRESSION IN RHABDOMYOSARCOMA
(Solid) Alveolar Rhabdomyosarcoma

myf4

- 71 pediatric rhabdomyosarcomas
- IHC for myogenin (myf4)
- <80% = “focal”
- >80% = “diffuse”
Diffuse staining for myogenin independent predictor of overall survival
INDIVIDUAL TUMOR TYPE-SPECIFIC PROGNOSTIC STUDIES

- 35 patients with extra-uterine leiomyosarcoma
- IHC for p53 and Ki-67

- No correlation between staining for p53 or Ki-67 and overall or recurrence-free survival
86 patients with primary localized synovial sarcoma
IHC for p53 and MIB-1
>10% cut-off for MIB-1
>25% cut-off for p53
p53 not associated with survival
MIB-1 index significantly associated with metastasis
• 49 patients with localized synovial sarcoma of extremities
• IHC for cell cycle-associated proteins, p53 and Ki-67
• >20% cut-off for Ki-67 (59% +)
• >10% cut-off for p53 (16% +)
p53 and Ki-67 associated with worse disease-specific survival

- 71 patients with myxoid liposarcoma
- IHC for p53
- >10% cut-off (17% positive)
Significantly higher metastatic rate for p53-positive localized tumors

- 32 patients with myxoid liposarcoma
- IHC for adipogenesis and proliferation-related proteins (RET, IGF1R, IGF2)
- >50% considered positive
Significantly lower metastasis-free survival for high expression of each
• 28 patients with leiomyosarcoma of deep somatic soft tissue

• IHC for c-myc
• >5% considered positive
Significantly lower metastasis-free and overall survival

- 88 patients with pleuropulmonary solitary fibrous tumor
- IHC for p53, various kinases
- >5% considered positive
p53
Multivariate analysis: p53 significantly associated with disease-free and overall survival
Gene expression profiling on 51 leiomyosarcomas

Unsupervised clustering: 3 clusters (1 “muscle-enriched”)

IHC on TMA for 5 markers with high mRNA in “muscle-enriched” cluster
a Unsupervised Hierarchical Clustering

Protein Expression of Group I/Muscle-Enriched Markers on LMS Tissue Microarray (n=377)

Strength of Staining:
- Strong
- Weak
- Negative
- Absent data

CASQ2
MYLK
CFL2
SLMAP
ACTG2
Multivariate model: muscle-enriched markers associated with improved survival, independent of site, grade, necrosis, and mitotic rate

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<th>Wald</th>
<th>HR (95% CI)</th>
<th>P-value</th>
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<tr>
<td>CSF1-response protein signature (Espinosa et al., 2009)</td>
<td>13.0</td>
<td>4.7 (2.0–10.9)</td>
<td>&lt;0.001</td>
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<tr>
<td>Number of positive group 1/muscle-enriched markers</td>
<td>4.5</td>
<td>0.77 (0.6–0.98)</td>
<td>0.035</td>
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<tr>
<td>Site</td>
<td>1.8</td>
<td>0.35 (0.1–1.6)</td>
<td>0.178</td>
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<tr>
<td>Mitotic figures</td>
<td>0.8</td>
<td>1.3 (0.7–2.4)</td>
<td>0.371</td>
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<tr>
<td>Grade</td>
<td>0.32</td>
<td>1.3 (0.6–2.5)</td>
<td>0.570</td>
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<tr>
<td>Necrosis</td>
<td>0.01</td>
<td>1.0 (0.5–2.2)</td>
<td>0.910</td>
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Final Comments I
Prognostic Role of IHC in Sarcomas

- IHC for accurate diagnosis (critical for outcome prediction)
- Other than diagnosis, grade remains key prognostic determinant
- Myogenic differentiation in pleomorphic sarcomas prognostic significance
- Other “biomarkers” not yet ready for prime time
Final Comments II
Prognostic Role of IHC in Sarcomas

- Studies of single tumor type (uniform histology, grade, etc.)
- Gene expression profiling identifying novel prognostic markers
- Goal will be to stratify patients into lower/higher risk groups – guide selection of therapy
Final Comments III
Prognostic Role of IHC in Sarcomas

• How to establish threshold for “positive”?  
• Need for uniform reporting  
• Need for pooled analyses/systematic reviews  
• How best to translate to clinical practice?
Over the past 25 years, immunohistochemistry (IHC) has played a central role in the classification of mesenchymal tumors. The chief contribution of IHC to diagnosis is to distinguish among histologically similar tumors, but IHC can also be applied (often for reassurance) to support the diagnosis of rare tumor types or to support the diagnosis when a tumor arises at an unusual location or in an unusual age group. The majority of the widely available IHC markers for sarcomas suggest lines of differentiation, such as smooth muscle actin and desmin for smooth muscle or myofibroblastic tumors, myogenin (myf-4) for skeletal muscle neoplasms, and S-100 protein for nerve sheath (Schwann cell) tumors. Unfortunately, few of these traditional markers are highly specific, and therefore a panel of markers is usually needed, and the results must be interpreted carefully in the context of the histologic and clinical findings. More recently, diagnostic IHC markers have been developed that can serve as surrogates for specific molecular findings, such as ALK for inflammatory myofibroblastic tumor, MDM2 and CDK4 for well-differentiated and dedifferentiated liposarcoma, TFE3 for alveolar soft part sarcoma, INI1 for malignant rhabdoid tumor and epithelioid sarcoma, and TLE1 for synovial sarcoma. However, IHC has no currently accepted clinical role in prognostication for sarcomas, beyond its role in establishing a specific diagnosis (which, along with grade, is among the most important prognostic factors).

Traditional prognostic markers for cancer in general, which provide critical information to oncologists both for counseling patients on the likelihood of developing metastases and
selecting appropriate systemic therapies, include such factors as tumor size, stage, and grade, and the presence of vascular invasion and lymph node metastases. In order for novel prognostic markers to gain acceptance in clinical practice, several broad requirements should be met: (1) the assessment must be reproducible and widely available with appropriate quality controls; (2) the marker must have substantial added value beyond that of established prognostic markers; and (3) the results of prognostic marker studies should be confirmed by additional independent studies. Prognostic marker studies can be classified into three general groups: phase I exploratory (hypothesis generating) studies that seek associations between potential markers and disease characteristics that have known prognostic importance, such as stage or grade; phase II exploratory studies that evaluate the ability of prognostic markers to discriminate between patients at high and low risk of disease progression or death; and phase III confirmatory studies that seek to validate the results of phase II studies using independent (preferably large and collaborative) data sets. These latter studies would ideally be prospective and protocol-driven, although systematic reviews or meta-analyses of phase II studies using pooled data are reasonable alternatives. Unfortunately, nearly all prognostic studies of IHC markers are phase II-type studies, without confirmatory follow-up studies using independent data sets. Indeed, it is uncommon for more than one study to evaluate a specific potential prognostic IHC marker.

In addition to the lack of critical confirmatory studies and systematic reviews, there are many other important issues that must be addressed for prognostic marker studies to yield clinically meaningful and applicable results. For example, how should continuous markers be dichotomized into "high" (or positive) and "low" (or negative) results? This question is particularly relevant to IHC studies, where the evaluated markers often show a range of staining in terms of both extent and intensity. Publication bias is also a significant issue for IHC-based prognostic studies, since such studies that yield negative results are rarely published, and, similarly problematic, published studies sometime omit the results of markers that failed to reach significance. Furthermore, multivariable regression models that include all known prognostic factors must be employed to determine whether the evaluated markers have independent prognostic significance. There are also additional problems that pertain to prognostic marker studies particularly relevant to sarcomas. Since sarcomas are very rare diseases, sample size is always an issue, and prospective studies are nearly impossible to perform. In addition, there can
be variability in both diagnosis and treatment of sarcomas among different cancer centers, pathologists and oncologists.

Many potential prognostic "biomarkers" have been evaluated in sarcomas, most in single or small numbers of retrospective studies. These range from "traditional" markers such as Ki-67 and p53, which have been studied for more than 20 years, to newer markers that are being identified through gene expression profiling. Ki-67 (or MIB-1) recognizes a nuclear antigen expressed in proliferating cells, preferentially in late G1, S, G2, and M phases of the cell cycle, but not in quiescent cells (G0). Ki-67 is widely used in pathology as a marker of proliferation, and, in some organ systems, as a criterion for malignancy or grading. The p53 gene encodes a protein that plays a key role in the cell cycle and apoptosis. p53 acts as a tumor suppressor gene; a wide variety of human cancers harbor loss-of-function p53 mutations. Since the wild-type p53 protein is rapidly degraded, IHC for p53 shows negative or weak staining in normal cells, whereas mutant p53 proteins usually have an extended half-life, and therefore tumor cells harboring p53 mutations usually show strong positive staining by IHC.

The "first generation" of prognostic IHC marker studies of sarcomas included all types (and grades) of tumors. These studies often reported conflicting results with regard to the prognostic significance of positive staining for p53 and/or a high Ki-67 index, and most failed to demonstrate independent prognostic significance of these markers in multivariate analysis. In contrast, more recent individual tumor type-specific prognostic IHC marker studies of sarcomas, including synovial sarcoma and myxoid liposarcoma, have demonstrated independent prognostic significance for expression of p53 and other IHC markers. Similarly, recent studies using gene expression profiling have identified groups of markers whose expression is associated with prognosis. These results have yet to be confirmed by other groups on independent data sets, and it is not yet clear how these findings can be translated to clinical practice.

Several retrospective studies have evaluated the prognostic significance of myogenic differentiation in pleomorphic sarcomas. In the first such study by Fletcher and colleagues in 2001, 100 extremity and trunk wall tumors formerly diagnosed as "malignant fibrous histiocytoma" were re-classified applying strict diagnostic criteria, in conjunction with IHC and
electron microscopy (in select cases). Upon re-review, the most common sarcoma types were high grade leiomyosarcoma and myxofibrosarcoma. In total, 30 of the tumors were classified as some form of high grade myogenic sarcoma. When the localized myogenic sarcomas were compared to non-myogenic tumors, the myogenic tumors showed a higher rate of metastasis. In a follow-up study by Deyrup and colleagues, 92 pleomorphic sarcomas of the extremities were immunostained for the myogenic markers smooth muscle actin, muscle-specific actin, desmin, and myoglobin; 42 tumors were positive for at least one marker. Similar to the prior study, myogenic differentiation was found to be an independent adverse prognostic indicator. Furthermore, there was an inverse relationship between the number of positive myogenic markers and survival. A subsequent study by Massi and colleagues re-evaluated 65 pleomorphic sarcomas of the extremities. Similar to the study by Fletcher, the most common diagnoses on re-review were leiomyosarcoma and myxofibrosarcoma; 31 tumors in all were some form of myogenic sarcoma. Upon multivariate analysis, myogenic differentiation was the only independent predictor of overall survival. These studies confirm the prognostic value of subclassifying pleomorphic sarcomas, for which IHC plays an important role, as well as demonstrate that pleomorphic sarcomas with myogenic differentiation (not only pleomorphic leiomyosarcoma and rhabdomyosarcoma, which are known to pursue an aggressive clinical course) have a higher metastatic potential. Such prognostic information can be helpful to select patients for clinical trials of novel chemotherapeutic agents.

Previous studies have shown that alveolar rhabdomyosarcoma typically displays diffuse staining for the skeletal muscle transcription factor myf4 (myogenin), whereas embryonal rhabdomyosarcoma (which has a better clinical outcome) usually expresses myf4 in only scattered cells. A recent study by Heerema-McKenney and colleagues evaluated the prognostic significance of the extent of myf4 staining in 71 pediatric rhabdomyosarcomas (>80% nuclear staining was defined as "diffuse"). Interestingly, the authors found that diffuse staining for myf4 was an independent predictor of overall survival, after adjusting for histologic subtype, anatomic site, stage, and age.

At present, IHC plays a limited role in prognostication for sarcomas, beyond supporting accurate diagnosis (which is a critical determinant of outcome). IHC is helpful to identify
pleomorphic sarcomas with myogenic differentiation, which have a higher metastatic risk than those without such differentiation. Other potential "biomarkers" are not yet ready for routine clinical application. Before the introduction of new IHC prognostic markers, thresholds for "positive" results will need to be examined, both in terms of biological relevance and so that the results can be reliably (and reproducibly) reported. Along these lines, there is a need for more uniform reporting of the results of these sorts of studies, to allow for systematic reviews including pooled analyses of data, so that novel prognostic markers can be validated and become a routine part of evaluation by surgical pathologists.

Key words: immunohistochemistry, soft tissue sarcomas, biomarkers, p53, Ki-67
Selected references:


Jensen V, Sørensen FB, Bentzen SM, Ladekarl M, Nielsen OS, Keller J, Jensen OM. Proliferative activity (MIB-1 index) is an independent prognostic parameter in patients with high-grade soft tissue sarcomas of subtypes other than malignant fibrous histiocytomas: a


