Clear Cell Sarcoma: A Historical Perspective with Personal Comments

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Recognition of a New and Distinct Entity

In 1965, Dr. Franz Enzinger published the first description of clear cell sarcoma of tendons and aponeuroses (9). His series of 21 cases was culled from the archives of the Armed Forces Institute of Pathology (AFIP) over a 25-year period. Two of the cases had previously been included in another publication from the AFIP on synovial sarcomas (2).

The cases described by Dr. Enzinger typically occurred in the foot and knee of young adults, especially women, and were intimately associated with tendons. The main symptom was a mass associated with pain in half of the cases, usually of long duration. The median age at onset of symptoms was 24 years, and the median age at initial surgery 26 years. All masses were solitary at presentation. No joint or bursal involvement was noted and bone erosion was seen in only one case. Tumor sizes ranges from 2 – 6 cm. (median, 4 cm.).

The salient histological features of the entity, illustrated in 7 of 21 cases, included fascicles and nests or cellular aggregates of pale, fusiform, epithelioid cells with clear to finely granular cytoplasm and round nuclei with very prominent nucleoli. The cellular aggregates were encased by delicate fibrous septa and were intimately associated with tendons and aponeuroses. Multinucleated tumor giant cells with 10 – 15 nuclei per cell were seen in two-thirds of cases. Mitoses were generally sparse and necrosis and hemorrhage were rare. Eight histochemical stains were performed; these showed intracellular glycogen in 8 of 15 cases and extra- and intracellular iron located primarily within dense fibrous tissue or septa. Intriguingly, small amounts of granular Fontana positive pigment not abolished by melanin bleach were found in 7 of 15 cases. The same areas were usually associated with hemorrhage and the pigment was also weakly positive with Gomori’s iron stain.

Follow-up information was available in 19 of 21 cases, with follow-up periods of 10 or more years in five cases and autopsies in six. Clinically the cases were characterized by a slow relentless growth, with repeated local recurrences and eventual metastases. Sixteen of 19 patients had local recurrences, usually within one year. Metastases occurred in 12 of 19 patients; all patients with metastases died of tumor. Fourteen of 19 patients died at a median interval of 4.5 years after diagnosis. Metastases were usually to regional lymph nodes and lungs, followed by the heart, liver and brain.

Prognostic factors were not clear at the time, aptly summed up by Dr. Enzinger’s comment that “…attempts to correlate the prognosis with the gross and microscopic features have been
unrewarding.” Survivals were shorter in larger tumors, but did not clearly correlate with prognosis. There was no clear correlation of outcome with mitotic rate or type of surgery.

Dr. Enzinger concluded that the cell of origin of clear cell sarcoma was unclear, stating that “…the term clear cell sarcoma of tendons and aponeuroses … is purely descriptive and reflects the uncertainty of histogenesis.” He further stated that it was not related to fibrosarcoma or synovial sarcoma (the most commonly rendered diagnosis), that it should not be categorized as tenosynovial sarcoma, and even more astutely, that it was not a spindle cell melanoma.

Forty years after its initial description, one cannot help but marvel at the precision with which this study was executed – on all levels, not to mention the creative genius involved in recognizing and accurately describing an entity that has withstood the scrutiny and analysis of time, several investigators, and ever evolving new technologies. The clinical histories are incredibly detailed; the completeness of the tables are exemplary with utmost attention to dates of treatment, local recurrences, metastases and treatment type; the results of intricate histochemical studies in the pre-immunohistochemical era are painstakingly documented and eerily accurate; and the follow-up is remarkably complete for a referral center (privacy laws hadn’t been implemented yet). The writing has a beautiful simplicity; it is clear and detailed yet concise. The measured nature of the conclusions regarding clear cell sarcoma’s histogenesis is a quality that runs through all of Dr. Enzinger’s original works; the balance between observation and conclusion is always rigorously scientific – never speculative or pontificating.

**Reaffirmation and Further Investigations Regarding Differentiation**

After the original detailed description of clear cell sarcoma, several small and large clinicopathologic studies appeared in the literature, reaffirming its existence and uniqueness as a clinicopathologic entity (6-8, 10, 13-15, 17, 18, 20, 27). Additionally, many of these addressed the histogenesis / differentiation of clear cell sarcoma, using first ultrastructural and later immunohistochemical techniques to investigate the mysterious Fontana positive granular material found within the cell cytoplasm. Thus, ultrastructural evidence of melanocytic differentiation was reported by several authors within one to two decades of the seminal description of clear cell sarcoma (1, 8, 12, 13, 16). This information was most clearly assimilated and cogently presented in 1983 by Kindblom et al. in their report of 15 cases of clear cell sarcoma (13). In six of the cases studied ultrastructurally, variable amounts of intracytoplasmic glycogen and external lamina surrounding groups of cells or individual tumor cells were seen. Bizarre were melanosomes found in one case. S100 protein was detected in all 15 cases. They concluded that clear cell sarcoma was indeed a homogeneous tumor entity of probable neural crest origin and not part of the nebulous group of tenosynovial sarcomas.

**Reassessment of the Clinical Course and Differentiation**

In 1983, Chung and Enzinger reported the accumulated experience of 141 cases of clear cell sarcoma retrospectively gathered from the archives of the AFIP from 1942 – 1980 (6). This study included the original 21 cases described by Dr. Enzinger in 1965 (7). Interestingly, the data had preliminarily been presented at the 67th Annual USCAP Meeting in Atlanta in 1978.
Although the follow-up period of this study was longer than the smaller series reported in 1965, the clinical findings in terms of tumor sites, presenting symptoms and sex distribution were similar. The median tumor size was less, as were the number of local recurrences, metastases and tumor deaths, probably due in part to the heightened awareness of the entity since its description in 1965. However, the authors pointed out that these tumors frequently developed recurrences and metastases 5 or more years after initial therapy, indicating the need for long-term follow-up with respect to treatment strategies.

New findings in this paper included the detection of intracellular melanin demonstrated in 72% of tumors with the more sensitive Warthin-Starry stain at pH 3.2. Again, iron and melanin were occasionally noted in the same cells. Additionally, intracytoplasmic glycogen was detected in two-thirds of cases. S100 immunostains were performed on a minority of cases and found to be positive in slightly more that two-thirds of cases. Notably, some of the cases of clear cell sarcoma bore a close resemblance to spindle cell melanomas of the ocular choroid, an observation that may well have been related to the presence of Dr. Zimmerman, the then current chairman of the Department of Ophthalmic Pathology at the AFIP.

In this paper, Drs. Chung and Enzinger stated that “…the presence of melanin was overlooked…”, not only by themselves, but by others. They capitulated to previous reports demonstrating melanogenesis in clear cell sarcoma, stating “…it is safe to conclude that: 1) clear cell sarcoma represents a malignant neuroectodermal tumor derived from potentially melanogenic cells that have migrated from the neural crest during embryonal life and 2) that the tumor is in many aspects akin to malignant melanoma and malignant blue nevus.” They thus concluded that a more appropriate term would be “…malignant melanoma of soft parts, rather than the purely descriptive term of clear cell sarcoma.”

Later, other authors stated that clear cell sarcoma was a preferable term, since clear cell sarcoma was clinically very unlike cutaneous melanoma (15). As we shall see, subsequent molecular genetic studies have confirmed this contention, illustrating the distinctness of the two tumors.

**Prognostic Studies**

Several relatively large studies on clear cell sarcoma were carried out within the next 25 -30 years following its description. The clinical features of some of the more significant studies are compared in Table 1. Oddly, studies focusing on prognosis were not performed until the statistical analysis published from MD Anderson Cancer Center in 1990 (27). Similar to Dr. Enzinger’s original study, no correlation between histological appearance and clinical outcome was found. Only tumor size ≥ 5 cm. was found to correlate with outcome (Table 2). Similar results were found in a Mayo Clinic series with respect to tumor size; any microscopic necrosis was also found to be an independent adverse prognostic factor (15). The following year, a rigorous statistical analysis of 58 cases that had not been previously reported from the AFIP was published (20). In that study, the authors found that larger tumor size as a continuous variable and any tumor necrosis were independent adverse prognostic factors. Interestingly, the tumors in their series were significantly smaller than those of other series (see Table 1), probably reflecting the relatively unbiased nature of the AFIP material seen at that time compared to major treatment referral centers. Again, no correlation between
clinical course, histological or immunohistochemical features, including proliferative activity, was found.

Subsequent prognostic studies have confirmed that size is the most important factor in the prognosis of clear cell sarcoma (7, 10, 17) and that radical surgery is the treatment of choice (10, 15). Chemotherapy still has little role to play in the treatment of this tumor (10).

Table 1. Comparison of Clinical Data of Major Studies on Clear Cell Sarcoma

<table>
<thead>
<tr>
<th>No. Cases</th>
<th>Sex Ratio M:F</th>
<th>Age (Yrs), Median &amp; Range</th>
<th>Tumor Size (CM), Median &amp; Range</th>
<th>% Cases with Follow-up / Median &amp; Range</th>
<th>Local Recurrences</th>
<th>Mets, Median Time to Mets &amp; Sites</th>
<th>% Patient Tumor Deaths &amp; Median Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>ENZINGER 1965</td>
<td>21</td>
<td>3:4</td>
<td>26 yrs 1 – 65</td>
<td>4 cm 2 – 6</td>
<td>95%</td>
<td>48 mos 1 – 36 yrs</td>
<td>84%</td>
</tr>
<tr>
<td>CHUNG &amp; ENZINGER 1983</td>
<td>141</td>
<td>6:7</td>
<td>27 yrs 7 – 83</td>
<td>3.3 cm 1 – 15</td>
<td>82%</td>
<td>68 mos (ave) 1 – 36 yrs</td>
<td>39%</td>
</tr>
<tr>
<td>SARA &amp; EVANS 1990</td>
<td>17</td>
<td>1:1</td>
<td>28 yrs 70 – 90</td>
<td>4.5 cm 2 – 9.5</td>
<td>100%</td>
<td>49 mos 3 – 158 mos</td>
<td>24%</td>
</tr>
<tr>
<td>LUCAS, NASCIMENTO, SIM 1992</td>
<td>35</td>
<td>2:3</td>
<td>30 yrs 10 – 64</td>
<td>4.5 cm 1 – 14</td>
<td>100%</td>
<td>74 mos 7 – 258 mos</td>
<td>14%</td>
</tr>
<tr>
<td>MONTGOMERY, RAMOS, MEIS ET AL. 1993</td>
<td>58</td>
<td>1:1</td>
<td>31 yrs 7 - 78</td>
<td>2.5 cm 0.6 – 9</td>
<td>84%</td>
<td>48 mos 2 – 264 mos</td>
<td>26%</td>
</tr>
</tbody>
</table>

Table 2. Adverse Prognostic Factors Affecting Survival in Clear Cell Sarcoma Patients

<table>
<thead>
<tr>
<th>Tumor Site</th>
<th>Tumor Size (CM)</th>
<th>Necrosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>SARA &amp; EVANS 1990 *</td>
<td>No</td>
<td>≥ 5 cm.</td>
</tr>
<tr>
<td>LUCAS, NASCIMENTO, SIM 1992 §</td>
<td>No</td>
<td>≥ 5 cm.</td>
</tr>
<tr>
<td>MONTGOMERY, RAMOS, MEIS ET AL. 1993 Δ</td>
<td>No</td>
<td>Yes, as a continuous variable</td>
</tr>
</tbody>
</table>

Table 2. Other factors analyzed but not found to be statistically significant:
*Age, sex, race, tumor site, symptom duration, initial therapy, mitotic rate, tumor necrosis, proportion of epithelioid cells, nuclear pleomorphism
§ Mitotic rate, nuclear pleomorphism, symptom duration, age, sex, tumor location and ploidy
Δ
Tumor size as a dichotomous variable, vascular invasion, presence of multinucleated giant cells, average and maximum mitotic rate, %PCNA positivity, immunostaining for HMB45 or S100 protein, age, race, sex, tumor site, pushing vs. infiltrative tumor margins, packeted vs. spindle cell patterns, tinctorial features of the cytoplasm (clear vs. eosinophilic)

**Cytogenetic and Molecular Genetic Studies**

The late 1980’s and 1990’s heralded the discovery of many tumor specific translocations for soft tissue sarcomas (19). The cytogenetic hallmark of clear cell sarcoma of tendons and aponeuroses is t(12;22)(q13;q12), resulting in a chimeric EWS/ATF1 gene in which the 3’-terminal part of EWS at 22q is replaced by the 3’-terminal part of ATF1 at 12q (3, 4, 14, 19, 21, 24 – 26, 28 – 30). This translocation has been detected cytogenetically in about 70% of reported cases of clear cell sarcoma. Many cases of clear cell sarcoma also display additional chromosomal aberrations including extra copies of chromosomes 8 and 7 and 2, and structural and numerical aberrations of chromosome 22 other than t(12;22) (3, 4, 26, 28, 29). To date, no cryptic translocations resulting in the EWS/ATF1 fusion transcript have been reported.

The EWS/ATF1 fusion transcript can be detected using RT-PCR and FISH techniques in about 50 – 75% of cases of clear cell sarcoma (14). Four types of EWS/ATF1 chimeric transcripts, designated types 1 – 4, have been identified in clear cell sarcoma and well characterized (21). These occur with different frequencies and sometimes simultaneously in a given tumor; type 1 seems to be the most common of these.

The histological and immunohistochemical features of clear cell sarcoma overlap significantly with those of cutaneous melanoma and occult metastatic melanoma, hence the differential diagnosis between these two entities still is problematic with profound clinical consequences (5, 23). Application of the aforementioned techniques in selected instances is potentially useful for definitive diagnosis.

Molecular genetic techniques have had an even greater impact on the ongoing controversy regarding the relationship of clear cell sarcoma to melanoma, and have unequivocally shown that clear cell sarcoma of tendons and aponeuroses is genetically distinct from cutaneous melanoma. Additional studies to detect mutations of exon 11 and 15 of the BRAF gene, which is commonly mutated in melanoma, are consistently negative in clear cell sarcoma (22). Thus, these tumors seem to develop through different genetic pathways.

Recently, t(12;22)(q13;q12) has been shown to not be unique for clear cell sarcoma of tendons and aponeuroses. We have recently reported a case of angiomatoid fibrous histiocytoma that displayed t(12;22)(q13;q12) as the sole cytogenetic abnormality (11). FISH, RT-PCR and sequence analyses revealed an EWSR1-ATF1 fusion gene that has previously been reported in clear cell sarcoma; no microphthalmia transcription factor (MITF-M) transcript was found. This study shows that:

1) The EWSR1-AFT1 chimera can be seen in tumors other than clear cell sarcoma, namely angiomatoid (malignant) fibrous histiocytoma.

2) The MITF-M transcript is not present in angiomatoid (malignant) fibrous histiocytoma, suggesting that its presence in clear cell sarcoma may be a reflection of its cellular origin rather than a consequence of transactivation by EWSR1-AF1 as proposed by others.
3) Activation of the EWSR1-ATF1 oncogene is probably an early step in the transformation process of clear cell sarcoma and other tumors. Overall gene expression patterns undoubtedly vary a great deal between different types of sarcomas with activation of EWSR1-ATF1.

**Concluding Remarks**

Forty years and many papers have passed by since the original description of clear cell sarcoma of tendons and aponeuroses by Dr. Enzinger. None of them have disproven his original observations or conclusions. This paper has withstood the acid test of time! Its uniqueness has been genetically confirmed. It is still viewed as a tumor of uncertain differentiation despite the detection of intracellular melanin.

Clear cell sarcoma of tendons and aponeuroses probably is the best name for this tumor. It has clearly been shown to be distinct from cutaneous melanoma. It is not to be confused by the uninitiated with other “clear cell” tumors, including clear cell sarcoma of kidney, clear cell myelomelanocytic tumor and clear cell carcinomas.

The original description of clear cell sarcoma of tendons and aponeuroses is, quite simply, a stroke of genius. It is one of many strokes of genius by Dr. Enzinger, who laid the first sound foundations for the classification of soft tissue tumors that have withstood the test of time, advanced medical technology, and critics. This paper also illustrates the remarkable gift that Dr. Enzinger has not only as a pathologist with superb observational skills, but as a physician with a broad perspective and as a superb writer. Dr. Enzinger is, without a doubt, one of the greatest figures in surgical pathology. His original works in soft tissue pathology will remain the indisputable gold standard by which future works are measured.
Bibliography


