Evolution of Pathological Staging and Histological Classification in Urological Malignancies

Ph.D. Thesis

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"Any classification system is only as good as

the existing methodology in determining the extent of the local tumour."

Donald G. Skinner

I. Introduction

In clinical pathology, the main aim of a tumour examination is to obtain information for the further treatment of the patient and to determine its prognosis. The tumour stage and histological type are the most important histopathological parameters and they provide the basis for a comparison of data obtain for different centers and for the creation of homogeneous patient cohorts to be used for therapy development. Historically tumour staging and efforts to achieve uniform histological classification commenced half a century ago [1]. The relevant literature is growing and it is continuously being restructured. Consecutive staging and histological classification systems exhibit significant differences, and appear quite unstable for the practising physician. These constant changes seem to endanger comparability over consecutive classifications, as well as the formation of homogeneous patient cohorts of high case numbers. It is also open to question as to whether these constantly updated classifications constitute a developmental process, or are only reorganisations of knowledge necessitated by the most recent information. To date it seems that there is no clear answer to this question in the available literature.

At the time of a decision regarding therapy or the introduction of new therapeutic procedures, it is well worth checking on the stability of the stage classification and histological subtype of the organ in question. If the classification (or some part of it) of a given organ is frequently changed, it is worth asking why this happens. One might ask, for instance, to what extent the frequent changes are due to statistical uncertainties or to methodical inadequacies.

II. Objectives

Our objective was to study the development of the two most important pathological factors required for the treatment of urological tumours, namely staging and histological typing, in order to obtain data to help devise more accurate procedures. We wished to identify those points where their application in daily diagnostic practice is the most problematic. Based on our knowledge investigations, two such areas were analysed in depth,

1. We wished to reduce the subjective component present in the pathological staging of radical cystectomies, and to this end we developed a cut-up protocol that minimises it.

2. We wished to examine the applicability of the genetically based classification of kidney tumours in the differential diagnostics of papillary kidney tumours.

III. Material and methods

The examination of the staging system evolution

The basis of pathological staging is the TNM (Tumour Node Metastasis) system created by the joint effort of the Union Internationale Contre le Cancer (UICC) and the American Joint Committee on Cancer (AJCC). It is in practice modified every five years on average, and so far (i.e. since 1968) it has been altered seven times. We evaluated the consecutive editions. It should be kept in mind that, although categories T, N and M have identical definitions in each edition (e.g. pT1, pT3a, N2), their meaning and content have often changed, shifted, been separated or merged, thus causing significant uncertainty in clinical practice. The results were evaluated graphically.

The examination of the radical cystectomies

From 2008 to the first quarter of 2012, 138 radical cystectomies were performed at the Department of Urology of the University of Szeged. These samples were histologically processed as a whole using macroblocks. With this method, only those samples were regarded as pT0 in which the granulomatous area and the hemosiderin deposition indicative of the earlier intervention were observable and the entire preparation was tumour-free. We called this technology as Gross Dissection Protocol for Radical Cystectomy (GDPRC). The samples were taken from 99 men and 39 women. The average age was 62.3 years; that of men was 64 years (range: 41-76), whereas that of women was 60.6 years (range: 49-71). As controls we used the data on 15,586 stages of 27,394 cystectomies presented in 15 publications from the period between 1971 and 2010. Here, we just concentrated on credibility. We reviewed those papers that described over 350 cases, demonstrated the practice, and each category was present, including pT0. Furthermore, the percentages were also given along with numeric data for each category.

We also created a summarised group from each category and counted the average frequency. The differences were analyzed with the one-sample z-test ($p < 0.05$).

The examination of the histological classification evolution

The standards in histological classification are set by the World Health Organisation (WHO), and are disseminated in the publications known in pathology as the "Blue Books". We examined these editions such as "Histological Typing of Urinary Bladder Tumours" was published in 1973, followed by "Histological Typing of Testis Tumours" in 1977, "Histological Typing of Prostate Tumours" in 1980 and "Histological Typing of Kidney Tumours" in 1981. Second editions on renal and testicular tumours were republished in 1998, on bladder tumours in 1999 and on prostate tumours in 2002. These already included immunohistochemical (IHC) photomicrographs. The two most recent WHO classifications are called "Pathology and Genetics. Tumours of the Urinary System and Male Genital Organs" (2004) and "Tumours of the Urinary System and Male Genital Organs" (2016) summarises urological tumours in one volume.

The genetic classification of papillary renal cell carcinoma

Papillary kidney tumours were chosen from samples of Department of Urology of the University of Pécs and Bács-Kiskun County Teaching Hospital in period from 1992 to 2012. They were taken from 6 men and 4 women. The average age was 64.7 years; that of men was 63 years (range: 54-68), whereas that of women was 65.6 years (range: 42-70).

Fluorescent In Situ Hybridisation (FISH) and Immunohistochemistry (IHC) examinations. Frozen sections were made from native tissues and placed on sylanised slides. Hybridisation using alpha satellite probes specific for chromosomes 7, 17, Y and 8, 12, 16, 20 (Vysis Downers Grove, IL, USA and Cytocell Ltd., Cambridge, UK) and post-hybridisation washing were performed in accordance the manufacturer's recommendations. In addition to the Y, 7 or 17 probe specific for chromosome was used as an internal control. The evaluation was carried out in a Zeiss Axioscope fluorescent microscope. A sample containing at least one hundred nuclei was examined and the percentage distribution was calculated.

IHC tests were performed on formalin-fixed, paraffin-embedded samples, making use of standard histopathological laboratory techniques.

IV. Results

The directions of evolution of the stage classification

When changes in the pTNM system of urological tumours are examined, it is apparent that the degree of changes is constant and continuous. However, pT and pN components are analysed separately (pM was practically unaltered), changes in pT appear to point towards some slight stabilisation. Changes in the classifications of testicular and bladder tumours have already shown a decreasing tendency in earlier editions, and the same can be observed for prostate tumours in the last edition. In contrast, the number of modifications in the classification of kidney tumours has increased in the last three editions and that of penile tumours in the last two editions. The stage classification of lymph nodes has displayed significant variations from the beginning, without any sign of stability, if we ignore the lymph node categories of testicular cancer in the last edition.

The advantages of GDPRC in radical cystectomies examination

The GDPRC has been applied as the daily routine processing procedure for all radical cystectomy specimens in our Department of Pathology since 2008. Up to March 2012, a total of 138 examinations were completed. The following emerged from and our series and the literature data: pT0 8.7 % and 6.1 %; pTa 0.7 % and 2.9 %; pTis 2.9 % and 6 %; pT1 15.2 % and 15.5 %; pT2 21 % and 23.3 %; pT3 34.8 % and 34.3 %; and pT4 16.7 % and 11 %, respectively.

Changes in the histological classification

The histological classification has undergone significant change in the past thirty years. New morphological, immunohistochemical, cytogenetic and genetic knowledge has led to an expansion of the number of entities. Tumours of the organs lying within the domain of urology should also be divided into benign and malignant groups. The border between these two groups, however, are not always distinct – a well-known example being the uncertainty surrounding the classification of papillary renal tumour, a relatively common tumour occurring as adenoma or carcinoma –, and histological diagnosis and determination of the histological subgroup is of decisive importance. Electron microscopic, immunohistochemical and molecular examinations have circumscribed types with different responsiveness to therapy and diverse prognoses.

A rare composite tumour: papillary renal cell carcinoma embedded in an oncocytoma

The Heidelberg-Rochester consensus, the first genetic assignment of kidney tumours had a major influence on the last two WHO classifications. In our published case the diagnosis of a combined tumour, a papillary renal tumour embedded in an oncocytoma was based on microscopic morphology and immunohistochemistry [2]. In certain regions the two tumour types were sharply divergent; in other foci they exhibited morphologies that were difficult to differentiate. To proceed further, we decided to apply immunohistochemical techniques. In view of the size and the structure of the lesion and the immunohistochemical pattern, the diagnosis was type 2 papillary carcinoma in an oncocytoma. No deviations were observed in FISH tests using centromer-specific probes for chromosomes 7, 17 and Y.

Studies on genetic disorders considered characteristic of papillary cell carcinoma

FISH tests on eight tumours diagnosed as malign on the basis of clinicopathological evidence produced mixed results. Only in two of the eight cases were the Heidelberg/Rochester classification criteria met, *i.e.* deviations supporting malignancy observed. In one case the genetic test only confirmed adenoma, even though malignancy was corroborated not only by the size but also by vessel invasion. In each of two other cases, only one chromosome was affected, which is insufficient for classification. In three cases, no deviations were detected.

Examination of the clonality of multiple papillary kidney tumours

Applying the genetic tests, papillary tumours are polyclonal, i.e. they emerge independently [3]. Our studies yielded the same result. Although the frequency of chromosomal deviations considered specific by the HC increased in proportion to tumour size, this was not coherent; polyclonality, however, was apparent.

V. Discussion

Besides the histologic type, the most important of a malignant tumour is the stage, since this is what will determine the post-operative treatment as well as prognosis. TNM-based staging will have been used for half a century and it has become widely accepted. One reason for its success is that it is easy to use and can be applied to all the different organs. Its general acceptance due to a broad-based consensus made possible the performance of clinical studies

with increasing case numbers and a comparison of different treatment procedures. However, it has to be applied with circumspection, because the TNM system is under continuous change, not only when new editions appear about every five years, but also due to the in-between revisions. (There have been three of these, namely in 1973, 1982 and 1992). During the comparison of consecutive issues, special attention should be paid to the fact that although the notation system of pTNM, i.e. pT, pN, pM and their subcategory labels do not change (with a few exceptions, such as the appearance of the V and S categories), the underlying content does. In other words, if earlier categories need be translated to the updated nomenclature, it may be impossible to do so. For example, a pN classification based on size cannot be compared to another based on the number of lymph nodes or their localisation.

The directions of evolution of the stage classification

Our study has revealed that the successive TNM classifications might be regarded as stations of a slow process of development. This is corroborated by the decreasing number of modifications in the description of primary tumours. Up until now, an increasing number of stages have retained their meaning over more and more editions. It is also of interest to look at the organs and stages that still display a considerable variation. This knowledge is useful not only for the researcher, but also for the practising physician, because it is not sufficient to know the rules – their meaning and limits also need to be clearly recognised. However, studies based on increasing numbers of cases provide little assistance in promoting development and resolving the uncertainties of stage classification; and small statistical shifts themselves should not be considered conclusive.

In our opinion, up to now the examination methods and their standardisation have received insufficient attention. The pathological stage is mostly determined by microscopy. The areas to be examined have to be selected, but this is done visually, retaining a significant subjective component in the examination method. One way to reduce this subjective factor may be to embed the entire organ and apply a suitable, accurate procedure along geometrical lines. Urology is fortunate in this aspect, because all the organs treated readily lend themselves to this approach. The necessary procedure was implemented some time ago for the prostate. The anatomical structures of samples derived from the kidney, the testicle and the penis are somewhat more complicated than those of the prostate, but still do not attain the complexity of a

cystoprostatectomy sample, representing seven organs (urethra, prostate, two seminal vesicles, bladder, and two ureters). We therefore applied a geometric approach to develop a cut-up protocol that was easy to use in everyday practice for the stage classification of radial cystectomy samples [4].

The advantages of the GDPRC in radical cystectomies

At present urinary bladder involvement by tumour is still being examined in a non-standardised fashion, depending to a large extent on the subjective assessment of the pathologist performing the cut-up. We identified this circumstance as a fundamental cause of the scattering of published pathological stage frequencies. Taking into consideration the above statement of Donald G. Skinner, doyen of American urology, we devised a protocol that allows the embedding of the entire sample. Based on 138 cases, we found that the findings accurately reflected the statistical average of 15,586 cystectomies. The deviations were due to more detailed processing: the case numbers of the pTis-pT2 group turned out to be lower and those in the pT3-pT4 group to be higher. In the case of pT4, the difference was significant (11.7% vs. 16.7% $p= 0.049$) [4]. We consider that the more frequent occurrence of cases in the highest stage may provide a partial explanation for the inaccurate prognosis of cystectomies because of under treatment.

Changes in the histological classification

The development of histological classification – unlike that of pathological staging – has been definitely driven by new methodologies. The initial light microscopic images have been supplemented by immunohistochemical data and, most recently, the discrimination of new entities has been made possible by genetic information, as for instance in the case of renal Xp11 translocation carcinoma. Since carcinomas are rooted in genetic disorders, it is sensible to classify them on the basis of genetic factors responsible for their development. Since these initial genetic factors cannot be corrected in the course of tumour evolution, they appear more coherent than images of the microscopic phenotype in the course of clonal propagation. Among urological tumours, a progressive, genetically based classification has so far been published for only one, in case renal tumour namely Heidelberg/Rochester classification.

Histological and genetic classification in the case of papillary renal tumours

Our intention was to utilise the HC in diagnostic practice, because the chromosomal deviations described by the HC appeared highly coherent and diagnostic [5, 6]. We reported our results at the Congress of the Hungarian Society of Pathologists, held in Gyula Hungary in 1998 [7]. Since our results did not fall into the accepted "mainstream", we did not attempt to increase the case numbers or publish our results. Nevertheless, neither the two WHO classifications following the HC, nor the last Vancouver Classification has adopted the differentiation of renal tumours based only on genetic differences. Chromosomal deviations that may be linked to renal tumours are listed, together with immunohistochemical data, as characteristics to support the microscopic image of the phenotype. Our own studies have also confirmed much the same thing. In the case of the papillary renal tumour embedded in an oncocytoma, its histological type was successfully verified in spite of the fact that it did not exhibit the chromosomal deviations that are characteristic of it. We were able to do this, because similar conclusions had been drawn in our earlier studies on papillary renal carcinoma and multiple papillary tumours. After realising that carcinomas are basically genetic diseases, great interest was focused on the genetic classification of renal tumours, embodied by the HC. Our findings are in general agreement with the data presented in the literature; however, as our genetic knowledge is at present still limited, more accurate information regarding tumour evolution will have to be collected.

VI. Summary

Stage and histological type are key histopathological parameters, but their consecutive classifications display significant differences. They vary and are unstable from a practical point of view. However, do the ever-newer classifications represent steps towards an evolution? Or are they merely a rearrangement of our knowledge supplemented by new, mostly statistical data as part of an endless process? We have not found a comprehensive study that investigated the development of the TNM system and the histological classification of urological tumours. Therefore we reviewed the successive editions of the WHO and UICC classifications. Our study has revealed that these might be regarded as stations of a slow process of evolution towards to the stabilisation. We though that improvement of methodology of staging and histology assessments, using a more precise procedures and modern examinations processes can accelerate this development.

This was why we introduced a new cut-up protocol of urinary bladder cancer to decrease pathologist subjectivity and we examined the applicability of genetic classification in the differential diagnostics of papillary kidney tumours. Nowadays both procedures are an integral part of our daily routine. Knowing their limitations, they prove to be valuable supports in uropathological work.

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II. ***Farkas Süköd****, Béla Iványi, László Pajor:*Accurate Determination of the Pathological Stage with Gross Dissection Protocol for Radical Cystectomy Pathology & Oncology Research July 2014, Volume 20, Issue 3, pp 677-685 **IF: 1.855**

III. ***Farkas Süköd,*** *Béla Iványi, László Pajor:* What can be more prognostic than the pTNM category assessed on radical cystectomy specimens? Virchows Arch. 2015 Oct;467(4):481-2. *(Letter to editor.)* *(IF: 2.627)*

IV. *István Sejben, Zoltán Szabó, Nándor Lukács, Márton Loránd,* ***Farkas Süköd****, Gábor Cserni :* Papillary renal cell carcinoma embedded in an oncocytoma: Case report of a rare combined tumour of the kidney. Can Urol Assoc J. 2013 Jul-Aug;7(7-8):513-6.
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