The Reticulin Algorithm for Adrenocortical Tumor Diagnosis: A Multicentric Validation Study on 245 Unpublished Cases.

THE RETICULIN ALGORITHM FOR ADRENOCORTICAL TUMORS DIAGNOSIS: A MULTICENTRIC VALIDATION STUDY ON 245 UNPUBLISHED CASES

Eleonora Duregon MD, Ambrogio Fassina# MD, Marco Volante MD, Gabriella Nesi^, Raffaella Santi^ MD, Gaia Gatti MD, Rocco Cappellesso MD#, Paolo Dalino Ciaramella°° MD, Laura Ventura ### PhD, Marcello Gambacorta °MD, Angelo Paolo Dei Tos§ MD, Paola Loli°° MD, Massimo Mannelli^^ MD, Franco Mantero## MD, Alfredo Berruti˜ MD, Massimo Terzolo MD, Mauro Papotti MD.*

Departments of Oncology and * Clinical and Biological Sciences, University of Turin at San Luigi Hospital, Orbassano, Torino, Italy. Department of Medicine (DIMED), # Pathology and Cytopathology Unit and ## Endocrinology Unit, and Department of ### Statistics, University of Padua, Padova, Italy. ^Divisions of Pathology and ^^Endocrinology, University of Florence at Careggi Hospital, Firenze, Italy. °Divisions of Pathology and °°Endocrinology, Niguarda Ca' Granda Hospital**,** Milano, Italy. § Department of Pathology and Molecular Genetics, General Hospital, Treviso, Italy. ˜Medical Oncology, University of Brescia, Italy.

Address for correspondence: Marco Volante, MD
Department of Oncology, University of Turin at San Luigi Hospital,
Regione Gonzole 10, 10043 Orbassano, Torino, Italy
Phone +390116705464; Fax: +390119026753; email: marco.volante@unito.it

Source of support: Work supported by grant from the Italian Association for Cancer Research (AIRC, Milan, grant no. IG/10795/2010 to MP) and Cariparo Foundation Excellence grant (Padova, 2011/2012).

Conflict of interest statement: All Authors declare the absence of any potential conflict of interest.

ABSTRACT

The pathological diagnosis of adrenocortical carcinoma (ACC) still needs to be improved, because the reknown Weiss Score (WS) system has a poor reproducibility of some parameters and is difficult to apply in borderline cases and in ACC variants. The “Reticulin Algorithm” (RA) defines malignancy through an altered reticulin framework associated to one parameter among necrosis, high mitotic rate or vascular invasion. This study aimed at validating the interobserver reproducibility of reticulin stain evaluation in an unpublished series of 245 adrenocortical tumors (61 adenomas and 184 carcinomas) from five italian centers, classified according to the WS. Eight pathologists reviewed all reticulin stained slides. After a training, a second round on discordant cases was performed ten weeks later. The RA re-classified as adenomas 67 cases (27%), including 44 with no reticulin alterations and 23 with altered reticulin framework but lacking the subsequent parameters of the triad. The other 178 cases (73%) were carcinomas according the above mentioned criteria. A complete (8/8 pathologists) interobserver agreement was reached in 75% of cases (k=0.702), irrespective of case derivation, pathologist’s experience and histological variant, and was further improved considering cases with high WS and clinically malignant behaviour, only. After the training, the overall agreement increased to 86%. We conclude that the reticulin stain is a reliable technique and an easy to interpret system in adrenocortical tumors; moreover, it has a high interobserver reproducibility which supports the notion of using such method in the proposed two-step RA approach for ACC diagnosis.

KEY WORDS: adrenal cortex, carcinoma, reticulin stain, reproducibility study, diagnosis.

INTRODUCTION

The pathological diagnosis of adrenocortical carcinoma (ACC) is based on the recognition of several morphological parameters, none of which is *per se* pathognomonic of malignancy. For this reason, they were combined in scoring systems that include up to 12 micro and macroscopical criteria.[1-4] These procedures are time consuming and hardly reproducible, unless used by experienced and specifically trained pathologists.[5,6] In the last decade, simplified versions of these scoring systems were proposed, grounded either on a different weight of a single parameter (e.g. mitotic rate and clear cells comprising 25% or less of the tumor in the Aubert's modified Weiss System)[7] or on an algorithmic approach centered on mitotic figure variability, such as the stepwise discriminant diagnostic system (SDDS).[8] The Weiss System (WS) is the most widely used diagnostic system nowadays, but its reliability is challenged in borderline cases, having only few criteria represented. Moreover, it is scarcely reproducible in the ACC morphological variants. In fact, specifically for the pure oncocytic neoplasms, a modified system has been proposed[9] while for the myxoid variant, some Weiss criteria are difficult to identify due to the peculiar morphology of such tumors.[10]

Some of us[11] have recently proposed a different approach, the "reticulin algorithm" (RA), which defines malignancy in adrenocortical tumors relying on a two step process: first the analysis of reticulin framework (highlighted by reticulin silver-based histochemical staining), then, if a disruption is recognised, malignancy is eventually defined through the identification of at least one of three malignancy-related criteria, i.e. necrosis, high mitotic rate (>5/50HPF) and venous invasion. This algorithm showed a similar diagnostic performance as compared to the WS, but it was faster and easier to apply. The efficacy of the RA was also confirmed in the rare oncocytic variant [12], whose diagnostic criteria are even more complex than those of classical ACC.

To date, the reticulin staining technique has only been used in a single report of 40 adrenocortical tumors[13] and quoted in a review article,[14] and still needs to be validated, at least with regard to the reticulin stain which is the first step of the algorithm. In fact, the second step is based on the identification of three parameters, which are part of the WS, and have been extensively reappraised in a recent reproducibility study by a French group.[6]

To this purpose, a multicentric validation study has been designed, aimed at assessing the reproducibility of the reticulin stain interpretation in a series of 245 newly collected and

unpublished adrenocortical tumors from five Italian centers, mostly including classical ACC forms and special variants, as well as a consistent number of benign tumors.

We here show that the reticulin stain is a fast and cheap technique, and an easy to interpret system in adrenocortical tumors, being both quantitative and qualitative changes considered in the evaluation of reticulin framework disruption, and that it has a high interobserver reproducibility, which supports the use of such simplified and fast method in the proposed two-step RA ("reticulin algorithm") approach for ACC diagnosis.

MATERIALS AND METHODS

Cases collection. 245 adrenocortical tumors were collected from the archives of the Pathology Divisions of five Italian Institutions and classified as adenomas or carcinomas according to the WS,[3,4] except for the 21 pure oncocytic lesions which were classified according to the system proposed by Bisceglia and coworkers,[9] as follows (**Table 1**):

a) 117 consecutive adrenocortical tumors having a WS ≥1 were collected between 2009 and 2012 from the pathology files of the University of Turin at San Luigi Hospital; this series included 19 cases resected at San Luigi Hospital and 98 surgical cases received in consultation from different hospitals. Among them, 13 cases were adenomas (ACA) with WS 1 or 2 (one had myxoid and another oncocytic features), while the remaining 104 were carcinomas with WS ≥3 (eight had myxoid changes, 17 oncocytic and one sarcomatoid features). The majority of these patients were treated at San Luigi Hospital in Orbassano-Torino, which serves as one of the referral centers for adrenocortical carcinoma in Italy. All previously published oncocytic and myxoid cases observed in this same period[10,12] and the 139 adrenal tumor cases originally described in the previous study on the RA[11] were excluded from the present investigation;

b) 61 adrenocortical tumors, including 26 ACA (two oncocytic) and 35 ACC (nine oncocytic and one myxoid) were recovered between 1993 and 2011 from the University of Florence at Careggi Hospital;

c) 26 ACA (four oncocytic) and 24 ACC (seven oncocytic and three myxoid) were retrieved from the pathology files of the University of Padua between 2000-2008;

d) one ACA and 14 ACC (three oncocytic and one myxoid) were retrieved from 1994 to 2007 at the Division of Pathology of Niguarda Ca'Granda Hospital, Milan;

e) three ACA and seven ACC (one oncocytic and one myxoid) were recovered from 1998 to 2012 at the General Hospital of Treviso, Italy.

Two cases, both carcinomas according to the Weiss system, were of pediatric age (a nine month female and a three year old male). These cases were also re-classified according to Wieneke's criteria,[15] as follows: one cases was classified as benign having 1/9 criteria (necrosis only) and the second as indeterminate for malignancy (3/9 parameters, including size, capsular invasion and necrosis).

The study received ethical approval from the local Review Board of our Institutions.

Histochemistry. To define the status of the reticulin framework, each Institution performed a reticulin histochemical staining on its own cases, using a commercially available silver impregnation-based kit (Bio Optica, Milan, Italy). The block for reticulin staining was selected based on the architecture of the tumor in H&E sections. Areas with a diffuse/nodular/trabecular (if present) rather that alveolar (normal adrenal-like) arrangement, which are morphologically consistent with reticulin disruption, were selected for reticulin staining.

All 245 reticulin stained slides were originally revised by a local pathologist (MP) in order to verify that a representative block was selected for each case, and re-classified according to the RA. In each slide, the reticular fibre framework was evaluated first at low (100x), then at high magnification (400x). The positive control was the normal adrenal gland on which we defined as "intact" the mesh appereance of reticular fibres, all with the same thickness, completely surrounding adrenocortical cells in nests or cords, a structure recalling an intact fishing net **(Figure 1 a-b)**. All patterns which differed from the reticulin network of a normal adrenal gland were recorded as "altered". Both quantitative and qualitative altered patterns were recorded. In the former case, the reticular fibre framework continuity was lost in more or less extensive tumor areas **(Figure 1 c-d)**, as previously described.[11] Qualitative disruptive changes were characterized by an apparently intact reticulin network made of fibers having variable and irregular thickness, with a frayed appearance, surrounding single cells or, more rarely, small groups of cells **(Figure 1 e-f)**.

To assess reproducibility of the reticulin stain interpretation, the same set of slides was circulated among eight pathologists with different experience in adrenal pathology (ED, MV, GG, AF, RC, APDT, RS, MG), in two rounds. During the first, participants (blinded of the original diagnoses) were separately asked to review and categorize the same 245 slides, choosing between

two possible options (normal or altered), based only on the description of reticulin patterns provided in our previous study.[11] In the second round ten weeks later, all discordant cases at the first screening (irrespective of the number of discordant observers) were re-assessed, without knowledge of the previous evaluation, after a training specifically designed on selected photomicrographs of 22 representative cases of different reticulin patterns at different magnifications.

Statistical analysis. Concordant diagnoses among the eight pathologists were computed, and non-parametric tests (Wilcoxon and Mann-Whitney) were used to test for differences in concordant diagnoses between subgroups and between the first and the second rounds. Fleiss's k statistics were used to assess agreement among the eight pathologists. Internal consistency of data was measured with the Cronbach's α statistic. Bootstrap methods were used to calculate 95% confidence intervals for the Cronbach's α statistic. Subgroup Fleiss's k and Cronbach's α statistics were also calculated. Statistical analyses were performed using the free software R (http://www.r-project.org/). A significance level of 0.05 was used.

RESULTS

Clinicopathological data. The main clinical and pathological features of the whole series of 245 tumors are summarized in **Table 2**. Follow up was available for 187 cases (including 39 adenomas and 148 carcinomas). 39 patients died of disease, 44 are alive with disease and 104 are alive with no evidence of disease (65 ACC and 39 ACA). These patients are currently on follow up at our Institutions with a mean follow up of 40 months (range 6-195 months for ACC and 6-96 months for ACA) .

Central reticulin stain analysis and Reticulin Algorithm application. Centralized evaluation of reticulin-stained slides showed that the stromal framework was similar to normal adrenal cortex in 44 cases (18%), whereas it was alterated in the remaining 201 cases (82%). The latter mostly included more or less extensive framework disruption, while a minority of them (13 cases, 6%, six of which of the oncocytic type) were rather charactherized by the qualitative alterations described in the Materials and Methods section. Interestingly, three cases (one oncocytic and two conventional) presented heterogenous reticulin patterns attributable to subclonal expansion

within otherwise normal adrenal-like areas **(Figure 2)**. Comparison with available Ki-67 immunohistochemical reactions of the same areas in two cases showed a significantly increased proliferation in reticulin disrupted as compared to preserved areas. All cases with altered network entered the second step of the algorithm and at least one parameter among high mitotic rate (>5/50HPF), necrosis and venous invasion was found in 178 cases. In the other 23 cases, no malignancy-related parameters were found in any of the hematoxylin and eosin stained available slides (from 5 to 13 per case). As compared to the WS and LWB scoring systems, six cases (all but two purely oncocytic), were classified as adenomas by RA rather than carcinomas as in the original diagnostic scoring, and all these patients are alive with no evidence of disease at current follow up (6-195 months, mean 49).

Interobserver reproducibility of reticulin stain evaluation. In the first round, the reproducibility of reticulin stain evaluation was performed on 245 cases by eight pathologists **(Table 3)**.

On the whole series and in the subgroup with follow-up available, the agreement rate among eight pathologists was 75%. Stratifying cases for WS, the full agreement was 24% in cases with WS<3 and 88% in those with WS≥3 ($p<0.0001$), whereas for clinical behavior was 61% in patients without evidence of disease and 92% for those who had a recurrence or died of disease, ($p<0.0001$). The "discrepancy" group (61 cases) was mainly constituted of tumors with a WS<3 (38/61, 62%) **(Figure 3)**. The kappa score calculated among all pathologists for the two possible options (intact versus alterated reticulin framework) was substantial ($k=0.702$), while the internal consistency of data was excellent using the Chronbach's alpha ($alpha= 0.954$). A subdivision of cases according to original Institution, pathologist's experience, ACC histological variant, WS and clinical behaviour indicated a satisfactory internal consistency of data through the Chronbach's alpha, while the kappa score may assume low values despite the high agreement because of the too small variability between and within raters. The two pediatric cases, that were discordant in terms of classification when considering Weiss or Wieneke's criteria, had both an altered reticulin pattern with a concordance at the first round of 7/8 and 8/8, respectively.

During the second round restricted to discordant cases at the first screening, performed blindly from the original assessment and after a specific training, a complete agreement among all eight pathologists was achieved in 44% of cases ($k = 0.62$, $alpha = 0.935$). Among them, all but four cases with a WS≥3 reached a complete agreement. The overall concordance rate following the second

round increased to 86%. Concerning cases with qualitative reticulin framework alterations, 5/13 were discordant in the first round, and an agreement of 13/13 was reached in the second round.

DISCUSSION

In this interobserver reproducibility study, the proposed RA approach for the diagnosis of ACC[11] has been validated with regard to the first step of such algorithm, specifically that based on the histochemical reticulin stain to detect the presence of reticulin fiber disruptive changes. The results of this study indicate that the RA: a) is a fast technique and easy to interpret system in adrenocortical tumors, when both quantitative and qualitative changes are considered in the evaluation of reticulin framework disruption; b) has a high interobserver reproducibility, which supports its routine use in the proposed two-step RA approach for ACC diagnosis.

Although such RA for ACC diagnosis is based on a two step procedure (reticulin disruption evaluation, followed by the recognition of three Weiss criteria, namely necrosis, mitotic count and venous invasion), we decided to restrict our reproducibility study to the first step of reticulin histochemical procedure since it represents a novel tool in the diagnostic procedure of adrenocortical tumors. In fact, although reticulin stain is a easy-to-perform ancillary staining recommended in the diagnostic practice of several pathological conditions including endocrine tumors (i.e. pituitary[16] and parathyroid[17]), it has not been validated in this specific setting, after the original observation by our group.

Therefore, the ability of different observers to identify reticulin framework alterations was assessed in a large series of unpublished adrenocortical tumors selected from five centers and including 184 ACC (Weiss score 3-9, with conventional, myxoid and oncocytic features), as well as 61 adenomas (Weiss score 0-2). To reproduce daily practice conditions, the five participant Institutions performed the reticulin histochemical stain in house and contributed the slides to build one single set of cases. This has been circulated and reviewed by eight pathologists with a different experience in adrenal pathology (two residents, two junior and four senior consultant histopathologists, all working in one of the participating centers). A substantial overall interpretation agreement was observed (75%, with a kappa value of 0.702) when each case has to be categorized between the two options of intact versus alterated reticulin framework, which is the base of the RA. Interestingly, the overall agreement and kappa values did not change significantly when a separate statistical analysis was done according to pathologists' experience

nor in the set of cases originated from different centers, thus confirming an acceptable reproducibility of the judgments among observers, among different sources of the tumor tissues, and among reticulin-stained slides prepared in different laboratories.

A detailed analysis of the 61 cases that proved to be discordant (even in only one out of eight observers) raised some issues that prompted a second blind review by each participant, preceded by a training using digital photomicrographs of representative cases and illustrating the intact reticulin framework of normal adrenal gland, cases with the "normal adrenal-like" mesh, cases with either quantitatively or qualitatively altered mesh and finally those with an extensively disrupted reticulin network.

The revision process allowed to confirm that cases assigned to the group of "normal adrenal-like" intact reticulin framework eventually were all adenomas according to the WS and were all clinically benign tumors and that cases having an extensive loss of reticulin fibers with marked disruption and fragmentation of the reticulin mesh were associated to the presence of one or more of the three Weiss criteria and ultimately were reported as ACC. However, within these two conditions, a fraction of cases (approximately 6%) had more complex reticulin patterns that during the first round caused some discrepancy in the various observers' interpretation, including 1) focally disrupted reticulin fibers in an otherwise intact tumor; and 2) an apparently intact reticulin network, surrounding single cells or small cell groups, but made of irregularly thickened fibers with markedly frayed borders, superficially resembling the reticulin framework of so called "hemangiopericytoma".[18] The former condition was more commonly observed in oncocytic adrenocortical tumors, while the latter was a feature of malignant tumors, in which qualitative (rather than quantitative) changes of the reticulin mesh were representative of the network disruption. This could reflect different growth and invasive properties of individual tumors in terms of connective tissue support to epithelial cells with either the absence or the reduction of the reticulin framework on one side or the production of a largely abnormal reticulin mesh occasionally surrounding single cells, thus mimicking a benign condition, but resulting in an irregularly tickened and frayed network. These observations expanded the proposed criteria for reticulin disruption evaluation[11] introducing both quantitative and qualitative analysis of the reticulin fibers. Incidentally, when the diagnostic algorithm was completed and compared with the classical WS based diagnosis and outcome, all such qualitatively disrupted tumors were classified as ACC. Moreover, although heterogeneity of reticulin staining profiles is frequent but usually presenting a mixture of different patterns (i.e. qualitative and quantitative changes), all ascribable

to disruption of the reticulin framework, three cases presented heterogenous patterns of staining suggestive of central subclonal expansion of a neoplastic population within otherwise normal adrenal-like areas at the periphery of the lesion, thus possibly supporting the hypothesis of an adenoma-to-carcinoma progression, as previously reported.[19]

The interobserver agreement after the second round raised from 75 to 86% (k=0.63, α=0.935), a figure significantly different from the original performance ($p<0.0001$) and supporting the notion that an accurate training in reticulin and Weiss criteria evaluation might help to further improve the diagnostic accuracy of ACC both in referral centers and in general surgical pathology divisions. In this respect, it is important to notice that a significant reproducibility was maintained also considering junior pathologists only, not specifically trained nor experienced in adrenal pathology.

Moreover, the reticulin histochemical stain worked well irrespective of the histological type. In fact, interobserver agreement was not significantly reduced in ACC variants, with special reference to the oncocytic one that was consistently represented in this series. This finding is of relevance because using classical scoring systems, myxoid tumors may be underdiagnosed due to the difficulty of identifying some Weiss parameters, while oncocytic adrenal tumors are easily overdiagnosed, due to the intrinsic occurrence of three Weiss parameters (eosinophilic cytoplasm, diffuse growth and atypias).[10,12,20,21]

Our detailed analysis of reticulin alterations and reproducibility of staining interpretation in a large cohort of adrenocortical tumors nicely correlates with a recent French reproducibility study of Weiss criteria designed on 45 adrenocortical tumors of the classical type (no oncocytic nor pediatric or myxoid cases were apparently included). In fact, all three Weiss criteria incorporated in the RA system proved highly reproducible among the different observers involved in the study.[6] In particular, necrosis was the most reproducible criterion with a “substantial” agreement (k value of 0.78) at first screening, that raised to an “almost perfect” agreement (k= 0.83) at the second reading round (after a dedicated training). The mitotic count performance significantly increased from a moderate to a substantial agreement after the training (k values of 0.54 and 0.65, respectively). Finally, with regard to the third parameter, venous invasion assessment remained a slightly more complicate task, with an observed moderate agreement (k value of 0.54), with no improvement after the second reading.[6]

In conclusion, the pathological definition of malignancy in adrenocortical tumors plays a relevant role in stratifying patients for further molecular studies and/or for specific treatments. Scoring systems or algorithms for assessing malignancy are the current popular diagnostic tools,

but need to be refined in order to have homogeneous interpretation criteria worldwide. This goal can be reached through the application of strictly controlled parameters, as demonstrated by the good reproducibility of Weiss parameter assessment after an accurate pathologists' training.[6] The same conclusion was reached in the current study in which a good reproducibility of the reticulin staining procedure and interpretation was obtained among eight observers from five centers with different experience in endocrine pathology. These findings are encouraging to implement the use of the RA, a fast, reproducible, cheap and easy to apply method proposed for ACC diagnosis.

REFERENCES

1. Hough AJ, Hollifield JW, Page DL, et al. Prognostic factors in adrenal cortical tumors. A mathematical analysis of clinical and morphologic data. *Am J Clin Pathol.* 1979;72:390-399.

2. van't Sant HP, Bouvy ND, Kazemier G, et al. The prognostic value of two different histopathological scoring systems for adrenocortical carcinomas. *Histopathology.* 2007;51:239-245.

3. Weiss LM. Comparative histologic study of 43 metastasizing and nonmetastasizing adrenocortical tumors. *Am J Surg Pathol.* 1984;8:163-169.

4. Weiss LM, Medeiros LJ, Vickery AL, Jr. Pathologic features of prognostic significance in adrenocortical carcinoma. *Am J Surg Pathol.* 1989;13:202-206.

5. Lau SK, Weiss LM. The Weiss system for evaluating adrenocortical neoplasms: 25 years later. *Hum Pathol.* 2009;40:757-768.

6. Tissier F, Aubert S, Leteurtre E, et al. Adrenocortical tumors: improving the practice of the Weiss system through virtual microscopy: a National Program of the French Network INCa-COMETE. *Am J Surg Pathol.* 2012;36:1194-1201.

7. Aubert S, Wacrenier A, Leroy X, et al. Weiss system revisited: a clinicopathologic and immunohistochemical study of 49 adrenocortical tumors. *Am J Surg Pathol.* 2002;26:1612-1619.

8. Blanes A, Diaz-Cano SJ. Histologic criteria for adrenocortical proliferative lesions: value of mitotic figure variability. *Am J Clin Pathol.* 2007;127:398-408.

9. Bisceglia M, Ludovico O, Di Mattia A, et al. Adrenocortical oncocytic tumors: report of 10 cases and review of the literature. *Int J Surg Pathol.* 2004;12:231-243.

10. Papotti M, Volante M, Duregon E, et al. Adrenocortical tumors with myxoid features: a distinct morphologic and phenotypical variant exhibiting malignant behavior. *Am J Surg Pathol.* 2010;34:973-983.

11. Volante M, Bollito E, Sperone P, et al. Clinicopathological study of a series of 92 adrenocortical carcinomas: from a proposal of simplified diagnostic algorithm to prognostic stratification. *Histopathology.* 2009;55:535-543.

12. Duregon E, Volante M, Cappia S, et al. Oncocytic adrenocortical tumors: diagnostic algorithm and mitochondrial DNA profile in 27 cases. *Am J Surg Pathol.* 2011;35:1882-1893.

13. Sanad S. Histopathological and Immunohistochemical Studies on the Adrenal Cortical Tumors of Egyptian Patients. *Life Science Journal.* 2011;8:8.

14. de Krijger RR, Papathomas TG. Adrenocortical neoplasia: evolving concepts in tumorigenesis with an emphasis on adrenal cortical carcinoma variants. *Virchows Arch.* 2012;460:9-18.

15. Wieneke JA, Thompson LD, Heffess CS. Adrenal cortical neoplasms in the pediatric population: a clinicopathologic and immunophenotypic analysis of 83 patients. *Am J Surg Pathol*. 2003;27:867-881.

16. Horvath E. Pituitary hyperplasia. *Pathol Res Pract.* 1988;183:623-625.

17. Papotti M, Volante M, Bussolati G. Applied Histochemistry in Disorders of the Endocrine System. In: Wick MR, ed. *Diagnostic Histochemistry*. University of Virginia, Charlottesville: Cambridge University Press; 2008.

18. Ambrosini-Spaltro A, Eusebi V. Meningeal hemangiopericytomas and hemangiopericytoma/solitary fibrous tumors of extracranial soft tissues: a comparison. *Virchows Arch.* 2010;456:343-354.

19. Bernard MH, Sidhu S, Berger N, et al. A case report in favor of a multistep adrenocortical tumorigenesis. *J Clin Endocrinol Metab.* 2003;88:998-1001.

20. Volante M, Buttigliero C, Greco E, et al. Pathological and molecular features of adrenocortical carcinoma: an update. *J Clin Pathol.* 2008;61:787-793.

21. Papotti M, Libe R, Duregon E, et al. The weiss score and beyond--histopathology for adrenocortical carcinoma. *Horm Cancer.* 2011;2:333-340.

FIGURE LEGENDS.

Figure 1. a: Reticulin framework of a normal adrenal cortex, in which reticulin fibres have a regular appearance, all with the same thickness, completely surrounding adrenocortical cells in nests or cords (40X, inset 200X); **b**: this pattern is reproduced in the reticulin network of an adenoma case (case #24 PD, 200X); **c** and **d**: quantitative changes in ACC with extensive loss and disruption of fibres (case #15 TO, **c** 100X, **d** 200X); **e** and **f**: qualitative alterations with irregularly thickened and frayed fibre in an apparently preserved mesh (case #59 TO, **e** 100X, **f** 400X).

Figure 2. A case of heterogeneous reticulin staining pattern (**a**) with normal adrenal-like peripheral areas (top of figure) colliding with a central nodule showing reticulin disruptive changes (bottom of figure); comparison with available Ki-67 immunohistochemical slide (**b**) showed a higher proliferation index in reticulin disrupted (bottom of figure) as compared to preserved (top of figure) areas (**a** and **b**: 100X; **insets** of b: 200x)

Figure 3. Distribution of reticulin staining interpretations (disruped in red, not altered in green) among the 8 parthologist in all cases, grouped according to the corresponding Weiss Score.

Table 1. Description of adrenocortical tumor cases enrolled in the study according to the center of collection.

	Total #245	Years of collection	ACA #61 (onco/myx)	ACC #184 (onco/myx)
Turin	117	2009-2012	13 (1/1)	104 (17/8)
Florence	61	1993-2011	26 (2/0)	35 (10/1)
Padua	42	2000-2008	18 (4/0)	24 (7/3)
Milan	15	1994-2007	1 (0/0)	14 (3/1)
Treviso	10	1998-2012	3 (0/0)	7 (1/1)

***Abbreviations*:** onco: oncocytic variant; myx: myxoid varaint of adrenocortical tumors.

Table 2. Comparison of clinical and pathological features of the present series and of that of a previous study on reticulin algorithm application.

	139 adrenocortical tumors (11)		**245 adrenocortical tumors (present series)**	
	ACA (47)	**ACC (92)**	**ACA (61)**	**ACC (184)**
F/M ratio	3.2	1.5	1.9	1.45
Age, mean [range]	53 [15-77]	46 [20-85]	54 [16-85]	48 [9 months-97]
Location L/R	23/24	48/45*	28/31	92/79^
Size, (cm) [range]	4 [1–10]	11.4 [1.6-25]	3.9 [1-12.9]	10.5 [2-30]
Weight, (g) [range]	39 [4–150]	428 [8-3100]	46 [2.2-201]	365 [5-2500]
Functional status				
not functioning	13	50	11	54
cortisol	18	13	13	30
aldosteron	15	6	8	4
androgens		8		8
cortisol+androgens				14
estrogens				3
not known	1	15	29	71
Status (outcome)#				
ned	47	32	39	63
awd		15		44
dod		28		39
recent cases		7		2
Weiss score				
0-2	47		61	
3-5		38		43
6-9		54		141
ACC variants				
Myxoid	-	6	1	16
Oncocytic	4	12	7	37
Sarcomatoid	-	-	-	1

***Abbreviations*:** ACA: adrenocortical adenoma; ACC: adrenocortical carcinoma; F: female; M: male; L: left; R: right; nf: not functioning; ned: no evidence of disease; awd: alive with disease; dod: died of disease; *: one case had bilateral tumor; ^: for 13 cases location was not specified in the original report; #: follow up data were available for 137 cases in the previous report (11) and for 187 cases in the current series.

Table 3. Interobserver agreement of reticulin staining in 245 adrenocortical tumors.

FIRST ROUND	# pathologist	# cases	Fleiss' K	Cronbach's alpha	Overall agreement
	8	all (#245)	0.702	0.954 (0.938, 0.956)	75%
	8	FI cases (# 61)	0.602	0.931 (0.894, 0.956)	59%
	8	PD cases (#42)	0.753	0.964 (0.941, 0.981)	71.4%
	8	TO cases (#117)	0.722	0.965 (0.938, 0.980)	83%
	4 junior	all (#245)	0.661	0.90 (0.868, 0.926)	88%
	4 senior	all (#245)	0.73	0.917 (0.881, 0.946)	78%
	7 exluding the one outlier	all (#245)	0.754	0.958 (0.941, 0.971)	84%
	8	WS ≥3 (#184)	0.325	0.816 (0.773, 0.883)	88%
	8	WS <3 (#61)	0.384	0.923 (0.887, 0.945)	24%
	8	classical ACT (#185*)	0.54	0.956 (0.939, 0.969)	77%
	8	purely oncocytic ACT (#26)	0.467	0.943 (0.883, 0.969)	58%
	8	mixed oncocytic ACT (#18)	0.486	0.933	83%
	8	myxoid ACT (#16)	0	0.546	81%
	8	dod+awd cases (#84)	0.281	0.816 (0.557, 0.890)	92%
	8	ned cases (#103)	0.529	0.954 (0.938, 0.967)	61%
SECOND ROUND	8	discordant in 1st round (#61)	0.62	0.935 (0.912, 0.954)	44%

***Abbreviations*:** FI, Florence cases; PD, Padua cases; TO, Turin cases; ACT, adrenocortical tumor; dod, died of disease; awd, alive with disease; ned, not evidence of disease; *including the only one sarcomatoid ACC.

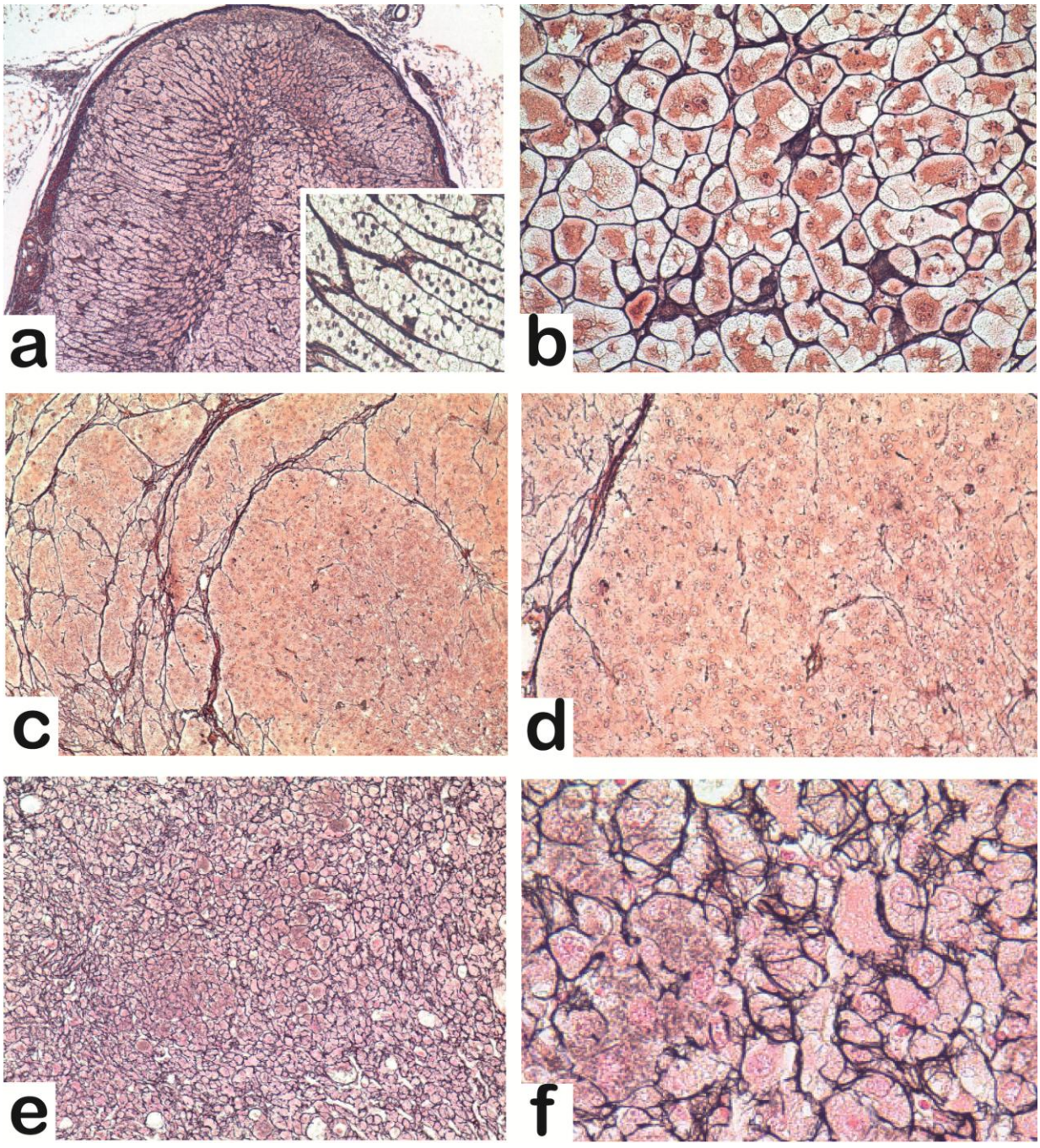

FIGURE 1

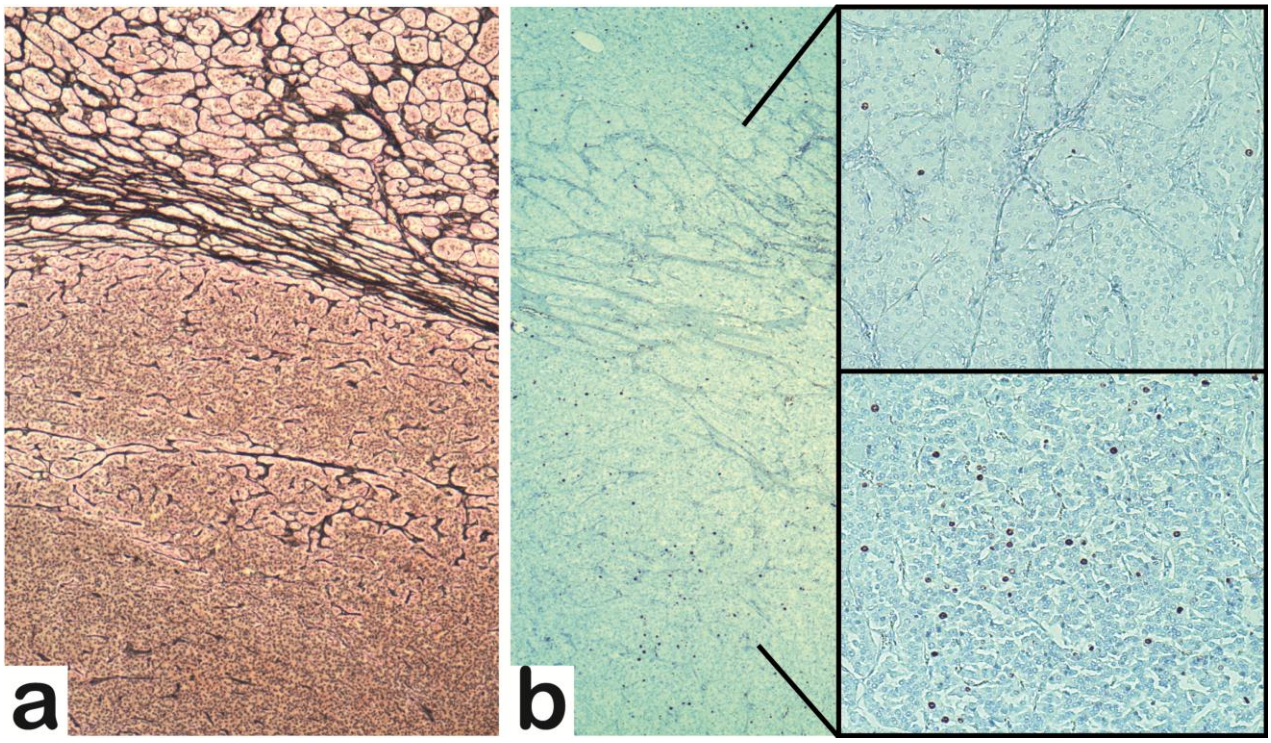

FIGURE 2

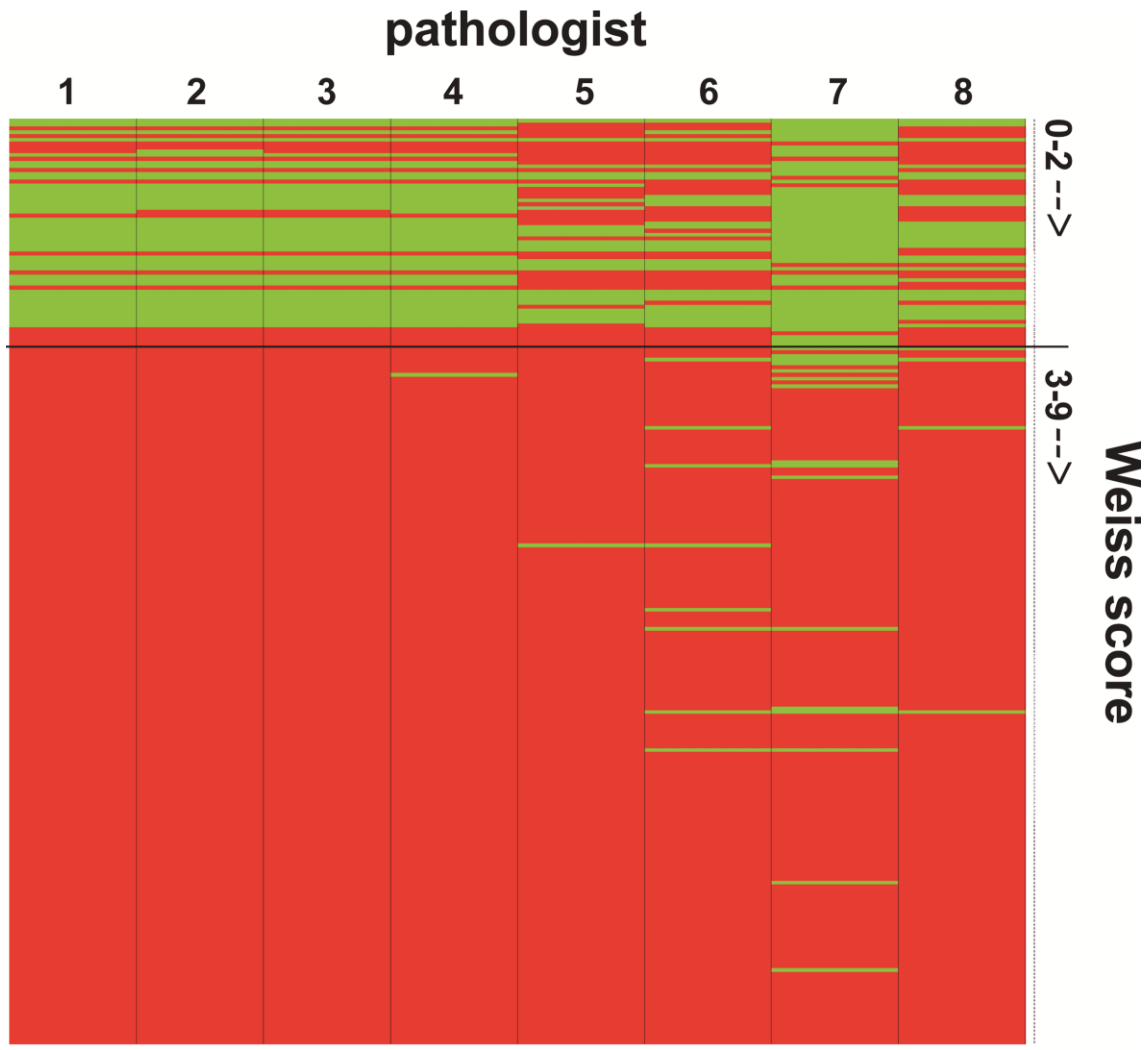

FIGURE 3