

Utility of Reticulin Algorithm in Diagnosis of Adrenocortical Tumors: A Re-visit

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ABSTRACT

Aim: This study aimed primarily at the assessment of the usefulness of the reticulin algorithm (RA) in the diagnosis of adrenocortical tumors in comparison with conventional multiparametric reporting systems in routine use.

Materials and methods: It was a retrospective study and included 40 adrenocortical tumors reported between 2013 and 2021 in the histopathology division of our institute, a tertiary care center in Kerala, South India. The demographic, clinical, and biochemical profiles, as well as macroscopic details of these cases, were recorded, and selected formalin-fixed paraffin-embedded blocks of the tumor were stained for reticulin fibers by Foot's modification. The two-step RA, which defines malignancy through a qualitative/quantitative alteration in reticulin framework along with at least one of the three histopathological parameters i.e., necrosis, venous invasion, and high mitotic rate, was applied to all tumors. The diagnosis thus obtained was compared with the original histopathology report based on the conventional scoring systems.

Results: Our cohort included 28 cases originally reported as adrenocortical adenomas and 12 as adrenocortical carcinomas (ACC) as per the classical scoring systems. On application of the two-step RA, all carcinomas were diagnosed correctly with diffusely altered reticulin framework. All but one case in the adenoma group were rightly classified as adenoma as per the RA as they failed to meet the two-tiered requirements for a diagnosis of carcinoma. The single discordant case was that of a huge adenoma with extensive hemorrhage, cystic degeneration, and necrosis, which showed only qualitative reticulin alterations probably attributable to the degenerative changes and lacked vascular invasion and high mitotic rate.

Conclusion: The RA provides a fast, cheap, and easy-to-apply method for the diagnosis of ACCs with good reproducibility and reduces dependence on multiple other morphological parameters included in conventional scoring systems, thus bringing down subjectivity. However, the procedure of reticulin staining is technically demanding, and extreme caution needs to be exercised at every step for good results.

Keywords: Adenoma, Adrenal cortical neoplasm, Carcinoma, Reticulin algorithm.

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INTRODUCTION

Reticulin fibers are type III collagen fibers that constitute the bulk of the supporting framework of cellular organs like the kidney, liver, and spleen. They are best demonstrated by silver impregnation methods with diagnostic applications in biopsies from various tissues. The adrenal gland is functionally differentiated into the outer cortex and inner medulla and is associated with the production of various hormones like aldosterone, cortisol, androgens, and catecholamines. It could harbor a variety of lesions, and adrenocortical tumors comprising adrenocortical adenoma and carcinoma constitute the major group. They warrant a correct and timely diagnosis for good outcomes.

While adrenocortical carcinoma (ACC) is a rare and aggressive disease with an annual incidence of 0.5–2.0 per million people, adrenal adenomas are increasingly being recognized owing to the availability of superior imaging techniques.¹ The diagnosis of ACC is often challenging owing to its rarity, wide range of morphological variations, and routinely used sophisticated multiparametric scoring systems. The reticulin algorithm (RA) was proposed by Volante et al.² in an attempt to define a simple yet highly specific and sensitive algorithm for the diagnosis and prognostic categorization of adrenocortical tumors. It defines malignancy through an altered reticulin framework along with at least one of the three histopathological parameters, that is, necrosis, venous invasion, and high mitotic rate.

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Here, we have retrospectively applied the RA to 40 adrenocortical tumors and tried to compare its efficacy in diagnosis against the complex scoring systems widely in use.

MATERIALS AND METHODS

A total of 40 adrenocortical tumors reported during the period from 2013 to 2021 were selected from the archives of the histopathology division of our institution, a tertiary care center in southern India. The slides and blocks of these cases were retrieved. The hematoxylin-eosin (H&E) stained slides were reviewed for the presence of the histopathological parameters, that is, necrosis, venous invasion, and high mitotic rate and appropriate blocks for reticulin staining

selected on the basis of the architecture of the tumor. Blocks with areas with diffuse/nodular/trabecular architecture that are morphologically consistent with reticulin disruption were chosen. These were carefully sectioned in the histopathology laboratory at 3–4 μm thickness, and staining for reticulin fibers was performed by the modified Foot's method.

The stained sections were evaluated for their reticulin framework, first under low power (100 \times) and then under high power (400 \times), to identify quantitative or qualitative alterations. The staining pattern of the normal adrenal gland was used as the control, that is, the mesh appearance of the reticular fibers, all with the same thickness, completely surrounding nests or cords of cells, referred to as the "intact fishing net" appearance. Any difference from this normal reticulin network was noted as "altered," and both qualitative and quantitative altered patterns were recorded.

The demographic details of patients, including age and sex, as well as weight, size, and the functional status of adrenocortical tumors, were obtained from the electronic medical records of the hospital. The histopathological features, including the Weiss/Lin Weiss Bisceglia scores, as well as the presence or absence of the three parameters for the application of the RA, that is, necrosis, high mitotic rate, and vascular invasion were recorded.

Compiling the findings of reticulin alterations and the three histopathological attributes, the cases were reclassified as per the RA into adenomas and carcinomas, and the results were compared with the original histopathology report. Thus, the efficacy of the RA in the correct categorization of the adrenocortical tumors into adenomas and carcinomas was assessed against the complicated multi-parameter scoring systems.

RESULTS

Of the 40 adrenocortical tumors included in our study group, 28 cases were originally reported as adrenocortical adenomas and 12 as ACCs as per the classical scoring systems.

Adrenocortical adenoma: This group included 28 cases with a diagnosis of adrenocortical adenoma as per the conventional scoring systems, including five oncocytic variants. The tumors had been classified based on the Weiss Score or Lin–Weiss–Bisceglia (LWB) criteria applicable to oncocytic variants. Accordingly, Weiss scores ranged from 0 to 2, while the LWB score was 0 for these cases.

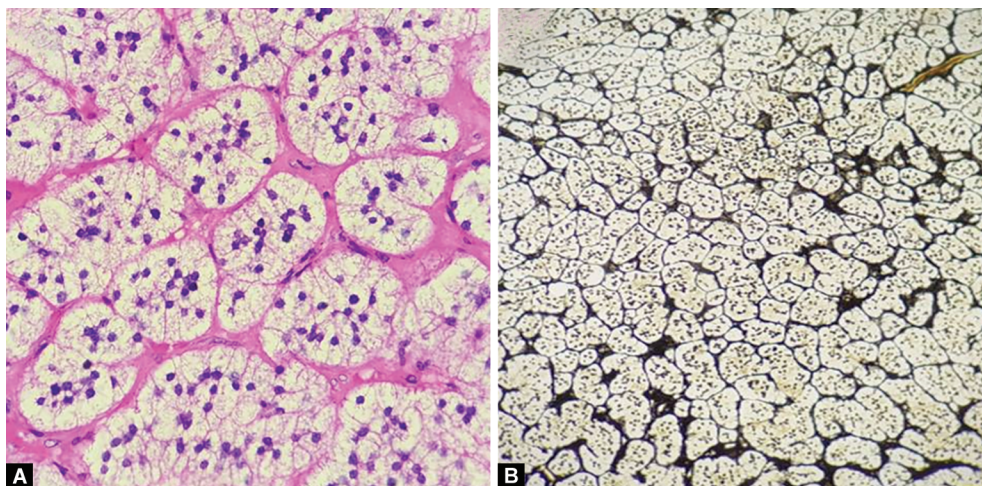
On the assessment of reticulin patterns, 18/28 adenomas showed mildly altered reticulin patterns as compared to normal adrenal glands, though mostly focal (Fig. 1). Among these, all but one (17/18) had quantitative alterations, that is, eight showed an increase in reticulin, and eight had decreased reticulin fibers. One case showed patchy areas of increased and decreased reticulin staining. Qualitative abnormalities were noted in eight, and seven out of them had coexisting quantitative defects. Only one case had an isolated qualitative defect. An important observation was that all five cases of oncocytic tumors had both qualitative and quantitative abnormalities, wherein pericellular condensation of coarse fibers was also observed.

In the second step of RA, out of the three histopathological parameters, none of the adenomas showed a high mitotic rate or vascular invasion. Only one out of the 28 adenomas showed necrosis, and it was associated with isolated qualitative reticulin abnormality. Thus, all adenomas other than the case with necrosis failed to qualify for a diagnosis of ACC as per the two-step RA and, hence, retained the adenoma diagnosis.

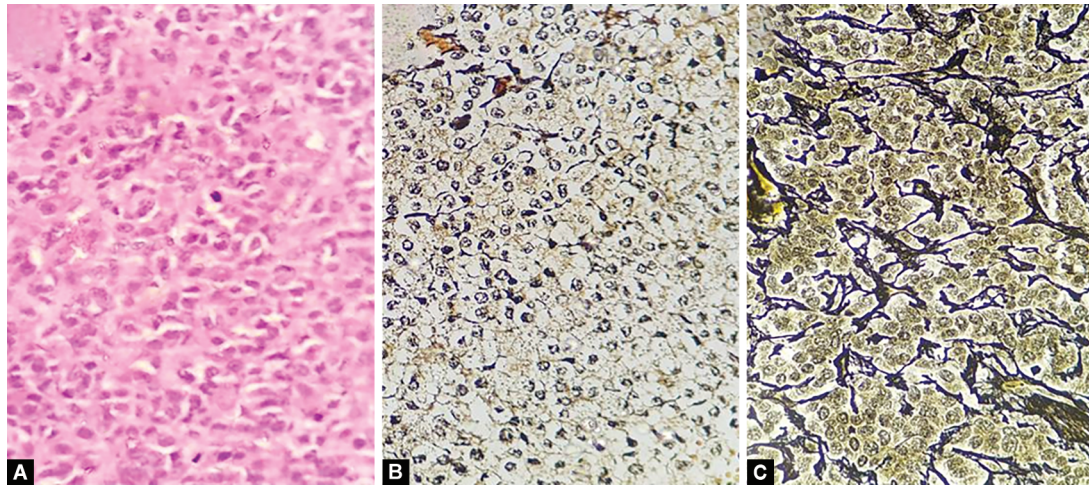
The adrenal adenoma cases had ages ranging from 7 to 67 years and included 17 females and 11 males. Their sizes ranged from 0.8 to 10 cm and weights from 5 to 400 gm. A total of 21 out of 28 were functional adenomas, the majority being associated with Conn's or Cushing's syndrome.

Adrenal cortical carcinoma: There were 12 cases with a diagnosis of adrenal cortical carcinoma, with Weiss scores ranging from four to nine, including one oncocytic variant. All 12 cases showed altered reticulin patterns (Fig. 2). A total of 10 out of 12 had quantitative changes with loss of reticulin in extensive tumor areas but included two who had areas of increased and decreased reticulin fibers. Seven had qualitative alterations with variable and irregular thickness of reticulin fibers as well as frayed appearance around single cells or small groups of cells. Five of these had coexisting quantitative abnormalities. The single oncocytic carcinoma had quantitative and qualitative alterations.

Among the histopathological features, 10/12 had mitoses $>5/50$ HPF. All 12 carcinomas showed necrosis, while eight/12 showed venous invasion. Out of the 12 carcinomas, seven cases had all three histopathological parameters, four showed two parameters, and one case had only one feature, that is, necrosis. Thus, all 12 cases qualified for a diagnosis of ACC as per the RA too.



Figs 1A and B: Adrenocortical adenoma—(A) H&E stain (400 \times); (B) Reticulin stain showing framework recapitulating normal adrenal gland



Figs 2A to C: Adrenocortical carcinoma—(A) H&E stain (400 \times); (B) Reticulin stain showing loss of reticulin in extensive tumor areas and (C) irregular thickness of reticulin fibers as well as frayed appearance

The adrenal cortical carcinoma cases had an age range between 36 and 75 years with a male-to-female ratio of 1.4:1. The size of tumors varied widely from 3.5 to 22 cm and the weight from 83 gm to a massive 2 kg. Only five out of the 12 carcinomas were functioning.

DISCUSSION

Adrenal lesions are often subtyped according to the anatomic site of origin, size, endocrinologic signature, morphologic features, or biological behavior. Over the years, the classification of adrenocortical tumors into adenomas and carcinomas has relied greatly on the classical multiparametric scoring systems like the Weiss score, the diagnostic performance of which has been exceptionally good ever since its introduction 27 years ago. Though it relies on well-defined and strict morphological criteria, its practical application is not always easy, particularly in borderline cases satisfying only one or two criteria or the morphological variants of carcinoma, especially among nonexpert pathologists.³

In 2009, Volante et al. endorsed the presence of a disrupted reticulin framework as a hallmark for the diagnosis of ACC, usually in more than half of the tumor. This has been attributed to the production by adrenal cancer cells of proteins involved in matrix digestion, such as matrix metalloproteinase type 2, 25, as well as the deregulation of gene transcription of laminin isoforms in malignant compared with nonneoplastic adrenal tissue. They observed that the most sensitive histopathological features were necrosis (recognizable in 84% of cases), followed by mitotic count $>5/50$ high power field (HPF) (71%) and venous invasion (64%). Thus, they came forward with the proposal of a simplified diagnostic algorithm with the identification of reticulin disruption as the primary step, followed by recognition of at least one of the unequivocal malignancy-related histopathological features (necrosis, high mitotic index, and venous invasion) as the second step. Their retrospective evaluation of a whole series of 139 adrenocortical lesions classified according to the Weiss system demonstrated that this RA was 100% sensitive and specific for recognizing all malignant cases.^{2,4}

Accordingly, in our retrospective study, which included 40 adrenocortical tumors, all 12 cases of ACC demonstrated more or less diffusely altered reticulin patterns with quantitative and/or qualitative abnormalities. All but one out of the 12 cases (91.7%)

were associated with at least two of the three histopathological parameters, as observed by Volante et al. in their study, wherein 84% of cases had at least two of the malignancy-related parameters together with reticulin framework abnormalities.² In keeping with their finding of necrosis as the most sensitive histopathological feature, 100% of our carcinomas also revealed necrosis, while high mitotic count and venous invasion were seen in 83.3 and 66.7% cases, respectively. Thus, in our study, all the carcinoma cases were correctly diagnosed by the two-step RA.

Among the 28 adrenocortical adenomas, we noted focal disruptions of the reticulin framework in 18 (64.3%). But, in the majority of these cases, the alterations were not very extensive. However, except for one case with necrosis, none of these portrayed any of the three classical histomorphological features, thus precluding a diagnosis of carcinoma. In an effort to validate the RA, at least with regard to reticulin staining, which is the first step, Duregon et al. conducted a multicentric study including 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. With the RA, they reclassified 67 cases (27%) as adenomas, among which 23 (34%) showed altered reticulin framework but lacked the subsequent parameters of the triad.⁵

While the focal decrease of reticulin fibers in adenomas could be attributed to bulkier acinar formations, all the oncocytic tumors showed diffusely increased and often pericellular reticulin fibers with qualitative changes, too. However, none but one of the adenomas qualified at the second step of RA for a diagnosis of carcinoma. Hence, as per our study, the second step of the algorithm is extremely important to prevent overdiagnosis of malignancy based on minor alterations in reticulin patterns. On review of the single discordant adenoma case, we noted that it was a huge 10 \times 10 cm sized tumor in a 48-year-old female with extensive hemorrhage, cystic degeneration, and necrosis, but showed only qualitative reticulin alteration probably attributable to the degenerative changes and lacked vascular invasion and high mitotic rate. On follow-up, at 7 years postadrenalectomy, she is doing well.

Adrenocortical carcinoma (ACC) is an infrequent malignancy with an incidence of 0.7–2.0 cases per million population per year. It can transpire at any age, with a higher incidence reported in females.⁶ In contrast, in our study, the male-to-female ratio was

7:5 (1.4). However, such a male preponderance was documented by Nair et al., too, with a male-to-female ratio of 1.17:1, in their single institution study from our part of the world, that is, Kerala, India.⁷ In our study, adrenal cortical carcinomas had an age distribution of 36–75 years with a mean age of 50.6 years, which was higher than that noted by Nair et al., that is, 46.8 years. However, the average age of presentation reported in the United States National Cancer Data Base is 55 years.¹

The true incidence of adenomas is not known as many are nonfunctional; however, estimates include 8.7% in the autopsy series and 4% in the radiology series⁸ with a female preponderance, as we noted in our cohort, too. Though classically described in the 50–70s of life, our patients belonged to a wide age group from 7 to 67 years.

Mukherjee et al.⁹ emphasized the importance of macroscopic examination as the first step towards the diagnosis of adrenocortical malignancy with a statistically significant correlation between the size/weight of the mass and the Weiss score. They described a weight >100 gm as the threshold for suspicion of malignancy. In their study, Stojadinovic et al. noted that 78% of carcinomas were larger than 10 cm in size.¹⁰ In keeping with these observations, the majority of our adrenocortical carcinomas also measured >10 cm in size and weighed well above 100 gm. On the other hand, about 90% of our adenoma cases fell short of these values.

Around 65% of all adrenocortical tumors included in this study were functioning ones. A total of 75% of the adenomas and 41.7% of carcinomas were associated with symptoms of hormone excess. In their study of adrenocortical masses, Mukherjee et al.⁹ observed that 47.4% of lesions were functional. However, Nair et al.⁷ reported only 27% of their carcinoma cases to be functioning, which they attribute to possible underdiagnosis of hormone overproduction. Also, according to the literature, functional adenomas are most commonly associated with hyperaldosteronism and Cushing syndrome, as we have noted in our study.

In adrenocortical tumors, the pathologic definition of malignancy is important for further molecular studies and specific treatment. In order to achieve uniformity worldwide, the diagnostic criteria should be based on the application of strictly controlled parameters with good reproducibility. On the technical front, reticulin staining is a demanding procedure wherein all factors, including tissue preparation, stability of reagents, staining time, pH, and temperature, are critical. However, the reticulin staining procedure and its interpretation have shown good reproducibility irrespective of the laboratory and the experience in the endocrine pathology of the center/pathologist, respectively. In the second step

of RA, the inclusion of only the three unequivocal histopathological features of malignancy, that is, necrosis, increased mitotic activity, and venous invasion, reduces the requirement of analysis of multiple other parameters which may be subjective, especially in centers lacking specific experience in these tumors. Thus, the RA provides a fast, cheap, and easy-to-apply method for the diagnosis of ACCs.

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REFERENCES

1. Wanis KN, Kanthan R. Diagnostic and prognostic features in adrenocortical carcinoma: a single institution case series and review of the literature. *World J Surg Oncol* 2015;13:117. DOI: 10.1186/s12957-015-0527-4
2. 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(5):535–543. DOI: 10.1111/j.1365-2559.2009.03423.x
3. Papotti M, Libè R, Duregon E, et al. The Weiss score and beyond—histopathology for adrenocortical carcinoma. *Horm Cancer* 2011;2(6):333–340. DOI: 10.1007/s12672-011-0088-0
4. Fonseca D, Murthy SS, Tagore KR, et al. Diagnosis of adrenocortical tumors by reticulin algorithm. *Indian J Endocrinol Metab* 2017;21(5):734–737. DOI: 10.4103/ijem.IJEM_573_16
5. Duregon E, Fassina A, Volante M, et al. The reticulin algorithm for adrenocortical tumor diagnosis: a multicentric validation study on 245 unpublished cases. *Am J Surg Pathol* 2013;37(9):1433–1440. DOI: 10.1097/PAS.0b013e31828d387b
6. Libè R. Adrenocortical carcinoma (ACC): diagnosis, prognosis, and treatment. *Front Cell Dev Biol* 2015;3:45. DOI: 10.3389/fcell.2015.00045
7. Nair LM, Jagathnath Krishna KM, Kumar A, et al. Clinicopathological features and outcomes of adrenocortical carcinoma: A single institution experience. *Indian J Urol* 2019;35(3):213–217. DOI: 10.4103/iju.IJU_19_19
8. Lerario AM, Moraitis A, Hammer GD. Genetics and epigenetics of adrenocortical tumors. *Mol Cell Endocrinol* 2014;386(1-2):67–84. DOI: 10.1016/j.mce.2013.10.028
9. Mukherjee G, Datta C, Chatterjee U, et al. Histopathological study of adrenocortical masses with special references to Weiss score, Ki-67 index and p53 status. *Indian J Pathol Microbiol* 2015;58(2):175–180. DOI: 10.4103/0377-4929.155308
10. Stojadinovic A, Ghossein RA, Hoos A, et al. Adrenocortical carcinoma: clinical, morphologic, and molecular characterization. *J Clin Oncol* 2002;20(4):941–950. DOI: 10.1200/JCO.2002.20.4.941