To the Editor.—The recent article by Washington and colleagues presents an updated protocol for the reporting of colorectal carcinomas (CRC) that is based upon the 6th edition of the American Joint Committee on Cancer (AJCC)/International Union Against Cancer (UICC) TNM Classification of Malignant Tumours for the staging of cancer. The 6th edition of the TNM Classification has attracted some critical comment with regard to CRC, mainly in relation to its proposals for the interpretation of subserosal tumor nodules as lymph node metastases or perivascular/perineural deposits. We would like to draw attention to an additional alteration in the current updated protocol, namely a change in the substaging of pT4 tumors in relation to previous recommendations and protocols. Surprisingly, this change is not discussed in the text or in the corresponding protocol recently published on the College of American Pathologists (CAP) Web site. Furthermore, in our opinion, there persists some confusion regarding the assessment of serosal/peritoneal invasion in CRC generally, which we would like to address.

On the first point, the most advanced histologic category for local invasion by CRC (pT4) has been subdivided previously into those tumors showing invasion of other organs or structures and those cases that involve or penetrate (previously termed perforate) the visceral peritoneum. In former guidelines these have been classified as T4a and T4b, respectively. In contrast, in the current report, direct invasion of other organs and structures is classified as stage T4b, while penetration of the visceral peritoneum is classified as stage T4a. No comment is made regarding this alteration and, therefore, it is not clear on what basis this adjustment to the staging system has been made. Previous guidelines and related review articles have commented that invasion of the visceral peritoneum indicates a worse prognosis than direct invasion of other structures. In our opinion, the evidence supporting this assertion is limited (as discussed in more detail below). However, if true, then it would seem more consistent with the principles of tumor staging that the more adverse finding (ie, visceral peritoneal invasion) would have the higher stage classification (ie, T4b). Thus, the new staging classification appears both unjustified and illogical.

More generally, since the substaging of T4 tumors has continued in the present protocol (albeit in reverse order), it may be worth reviewing the evidence that this distinction is of clinical significance. It is now widely accepted that visceral peritoneal invasion in CRC is associated with adverse prognosis in CRC, although there remain difficulties in diagnosis and this feature may be underdiagnosed by histopathologists. In contrast, few studies provide specific information on the prognostic significance of tumor invasion of other structures or organs in the absence of concurrent serosal invasion (ie, stage T4a, as defined by the previous staging guidelines). While such cases undoubtedly occur (the example chosen for illustration in the present protocol is a tumor directly invading the cecum), in our experience, this type of invasion is very uncommon. Review of a database comprising 378 consecutive resections for CRC accessioned in our institution showed 73 (19.1%) stage T4 cases. Of these, 67 were stage T4b (serosal invasion, when using the previous nomenclature) and only 5 cases (6.9% of T4 cases or 1.3% overall) were classified as stage T4a. Thus, based upon our experience, it would require a very large series to prove any difference in prognosis between stage T4a and T4b tumors. It is also worth noting that the survival data used to support the T4a/4b distinction provided in the 1993 TNM Supplement (and often quoted in subsequent protocols) were based upon unpublished work and that the text commented that the differences in survival were not statistically significant. To our knowledge, more recent studies that have addressed the prognostic significance of local invasion in CRC have not subdivided stage T4 cases. Therefore, it is not possible to compare outcomes between cases with serosal involvement and those with only direct (nonserosal) invasion. This weakness of the current TNM staging system was noted by the Association of Directors of Anatomic and Surgical Pathology in their recommendations for the reporting of resected CRC.

We would also like to comment on apparent inconsistencies in the histologic definitions of peritoneal invasion. The current and previous protocols refer to the detailed studies of Shepherd and colleagues and Petersen and colleagues, in which the authors described 4 patterns of local peritoneal involvement (LPI). Group 1 LPI was defined as tumor well clear of the closest peritoneal surface; group 2 LPI as mesothelial inflammatory/hyperplastic reaction with tumor close to but not actually present at the peritoneal surface; group 3 LPI as tumor present at the peritoneal surface with inflammatory reaction/mesothelial hyperplasia/“ulceration”; and group 4 LPI as tumor cells demonstrated free in peritoneum and with evidence of adjacent “ulceration.” These patterns were termed grades rather than groups in a recent review article. While these definitions have been very useful, in our opinion, the terminology has proved somewhat confusing since LPI groups (or grades) 1 and 2, as defined above, were considered by Shepherd and colleagues to be negative for peritoneal invasion. Furthermore, although the data provided in the studies indicate that only LPI groups 3 and 4 were regarded as “true” peritoneal invasion, this was not explicitly stated in the text. This may explain the conflicting interpretation of peritoneal invasion by other authors referring to the above studies. For example, previous protocols and corresponding review articles that referenced the work of Shepherd et al state that serosal involvement by CRC included 3 types of local peritoneal involvement, all of which were said to be associated with a shorter survival (italics added). These 3 types corresponded to the LPI groups 2 to 4, as originally defined by Shepherd and colleagues and Petersen and colleagues; in oth-
er words, tumor well clear of the serosal surface, or LPI group 1, was omitted. However, this point was not clarified, and thus, confusingly, peritoneal invasion types 1 to 3, as outlined in the protocols, equated to LPI groups 2 to 4. Furthermore, peritoneal invasion type 1 (tumor close to the serosal surface with inflammatory and/or mesothelial reaction, or LPI group 2) was initially regarded as positive for serosal invasion. Later, this issue became more blurred, since it was recommended that “the diagnosis of T4b encompass at least types 2 and 3 of serosal involvement [italics added],” in other words, LPI groups 3 and 4. The current protocol is more definite in recommending that “the T4a category encompasses types 2 and 3 of serosal invasion.” However, while this appears more consistent with the original data of Shepherd et al and Petersen et al, the text continues to state that “all the previously listed types of peritoneal involvement (ie, types 1–3 or LPI 2–4) were associated with decreased survival.” We believe that this comment is not supported by the data, at least not that presented in the studies that are referenced in the current protocol. Recently, it has been suggested that if tumor is within 1 mm of the free peritoneal surface (probably corresponding to many Shepherd LPI group 2 cases), then this should be regarded as an indication of possible transperitoneal spread but staged as T3. Updated protocols, such as the one presented by Washington et al, are very useful to all pathologists interpreting and reporting upon CRC resection specimens. Nevertheless, like Quirke and colleagues, we believe that changes to staging classifications should be justified and that the data, purportedly supporting previous assumptions, should be critically scrutinized. Hopefully, where relevant, this may provide the impetus for additional investigations that ensure that modifications to future staging systems are based upon solid data.

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The authors are most grateful to Professor C. Wittekind for providing information on the 1993 TNM Supplement.


In Reply.—The authors wish to thank the readers for their comments regarding the new College of American Pathologists (CAP) Colorectal Check-List Protocol. We understand the concerns regarding the apparently arbitrary change in the substaging of pT4 tumors, as articulated in your letter and in a recent letter published in the ARCHIVES® and realize, in retrospect, that it was premature to change the T4 categories in this current checklist. The change to classify tumors with direct invasion of other organs as T4b and those with involvement of the visceral peritoneum as T4a is based upon a population-based analysis of more than 100,000 colon cancer cases conducted by the Hindgut Task Force of the American Joint Committee on Cancer (AJCC) and reported in the 2008 American Society of Clinical Oncology (ASCO) annual meeting. These data indicate that, for each category of N, the new T4a category is associated with a 10% to 20% better 5-year survival for affected individuals than that associated with locally invasive carcinomas. This analysis forms the basis for proposed changes to TNM classification for colorectal cancer for the 7th edition of the AJCC Cancer Staging Manual, which is currently scheduled to be published in late summer of 2009, and the International Union Against Cancer (UICC) agreed in 2008 to these proposed changes to T4a and T4b.

Regarding the inconsistencies in histologic definitions of peritoneal invasion, we appreciate the summary of the literature on this topic. We agree that assessment of local peritoneal invasion may be difficult and that accuracy of the diagnosis of serosal involvement is dependent on the quality of the assessment tech-
nique. It has been recommended that at least 2 blocks be taken from the area where the tumor is closest to the serosal surface.4,5 If tumor extends close to the peritoneal surface without actual involvement of the surface, multiple tissue levels through the block may be justified to confirm or exclude possible involvement. Underdiagnosis may be avoided by cytology techniques such as serosal imprint cytology.6 Positive peritoneal cytology may be found in as many as 25% of cases without identified serosal involvement on routine histology, which would otherwise be classified as pT3.

While we agree in principle that tumor within 1 mm of the serosal surface should be assigned to the pT3 category, the recent review and other publications7 by Shepherd and colleagues8 offer support for classification as pT4 in situations in which tumor is continuous with the serosal surface through an area of inflammation, such as in cases with sigmoid carcinomas involving diverticular disease. We agree that further data from carefully conducted studies of local peritoneal involvement are needed to refine future staging systems.

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Examination of Specimens From Patients With Ductal Carcinoma In Situ of the Breast Using Large-Format Histology Sections

To the Editor.—The College of American Pathologists published the recommended protocol for examination of specimens and reporting ductal carcinoma in situ (DCIS) of the breast in the January issue of the Archives of Pathology & Laboratory Medicine.1,2 The protocol was partly based on the results of 2 independent studies comparing different histology methods for tissue sampling in excision specimens with DCIS that were published in the same issue.3,4 The tested methods in these studies tended to substantially underestimate the extent of the disease.

There is another histology method that was not mentioned which—as adapted to the needs of diagnostic routine—is more accurate for assessing the extent of carcinoma in situ of the breast than the tested ones.5,6 Using large-format (10 × 8 cm) contiguous histology sections enhances mammographic pathologic correlation,6 documents the lesion for adequate and reproducible analysis of the extent and distribution of the disease,7 and preserves the relation of the lesions to each other and to the circumferential surgical margin. As we have demonstrated previously, this method allows us to delineate extensive and nonextensive DCIS, where the categories correlate significantly with the rate of local recurrences.8,9

Several laboratories in Europe explore the advantages of large sections in diagnosing breast carcinoma. Because a few laboratories in the United States have also adopted this technique, it would be of value to include it into the protocol, at least as a possible alternative.

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Coronal Serial Sequential Sampling of Breast Specimen in the Assessment of the Extent of Ductal Carcinoma In Situ

To the Editor—We read with interest the articles of Dadmanesh et al1 and Grin et al.2 As pointed out by the authors, the extent of ductal carcinoma in situ (DCIS) is an important prognostic factor for local recurrence after segmental resection. As noted, the method of measurement based on the mammographic findings is often inaccurate. Measuring directly from the slide is appropriate if the lesion is present in its entirety on a single slide. This is usually not the case, however, and there is as yet no standard method for measuring the extent of disease in more extensive lesions. The authors compared the number of involved blocks method with the “gold standard” method, in which specimens were serially sectioned perpendicular to the long axis of the specimen and then submitted in toto. Sectioning breast mastectomy and segmental specimens using this technique tends to divide the region of DCIS involvement into multiple segments. We believe this makes the measurement of the size of DCIS more difficult and prone to error.

As reported in our previous studies, we have had occasion to examine breast specimens (both mastectomy and segmental resection) by coronal sectioning (ie, parallel to the chest wall).3,4 In this technique, each sequential section had a thickness of 5 mm and resulted in the largest surface possible per slice. The grossly suspicious regions of these large coronal sections were submitted for microscopic examination by dividing the area in a grid pattern. When cut this way, the tissue blocks were also in the coronal plane. The assembled glass slides from each plane of coronal section will likely represent the largest section of DCIS in that plane of section. When no gross lesion is discernable, the sampling of multiple regions without destroying the anatomic relationship of the remaining tissue is possible when the sections are large and intact. This facilitates revisiting the gross specimen to take additional samples from regions of microscopic involvement.

Ductal carcinoma in situ is a preinvasive malignant lesion typically involving the one major duct and its branches, as demonstrated by a 3-dimensional study.2 The process most often involves the duct system by continuous or discontinuous spread (with skipped areas of uninvolved duct) along the duct and its attributor branches and acini. Occasionally, DCIS involves more than one duct system, thought to be due to either true multicentric origins or via spread to the nipple skin and secondary extension into other duct system. We believe that coronal serial sequential sampling offers the following advantages:

1. Increased likelihood of obtaining a panoramic view of the lesion in a single or small number of slices, facilitating exact localization of the DCIS and measurement of the greatest diameter of the lesion.5,6

2. Easier mapping of sections taken for microscopic examination.

3. Easier correlation with perimeter margins as viewed from a coronal plane (ie, left/right, medial/lateral, inferior/superior).

4. Ability to go back to the tissue area of interest not yet submitted for microscopic examination.

5. Easiest direct correlation with magnetic resonance imaging coronal views of the breast.

With respect to the superficial and deep resection margins not visualized in a coronal plane, serial sections perpendicular to the inked resection margins of all suspicious areas should be taken.

The authors have no relevant financial interest in the products or companies described in this article.


Erroneous Detection of Hypercalcemia in Specimens Stored in Greiner Bio-One Vacuette Plasma Separator Tubes and Analyzed by the Arsenazo III Methodology

To the Editor.—Assay interferences from blood collection tubes have been well documented in a number of studies.1–3 Bowen et al1 compared a number of analytes in samples obtained in SST (BD Diagnostics, Franklin Lakes, New Jersey), glass tubes, and Vacuette tubes (Greiner Bio-One, Monroe, North Carolina) and measured on an IMMULITE 2000 analyzer (Siemens Medical Solutions Diagnostics, Deerfield, Illinois). They found a clinically significant bias in total triiodothyronine (T3) results in samples collected in SST tubes. In another study, gel tubes were found to not be suitable for blood collection for analysis of tricyclic antidepressants because of absorption of the drugs by the gel.2 These interferences may not be detected by standard quality assurance measures because quality controls and proficiency test samples are not stored in the tubes that are used for patient blood collection.4 We identified an interference that is estimated to have caused erroneous claims of hypercalcemia in 5% of all total calcium laboratory reports. This may be clinically significant, since elevated total calcium in an older patient frequently prompts further investigation for underlying malignancies. In our experience, the interference is observed only with the following constellation of factors: collection in Vacuette Plasma Separator Tubes (product number 454008, Greiner Bio-One), delay of testing, and analysis by total calcium method on Architect c8000 analyzer (Roche Diagnostics Corp, Indianapolis, Indiana). When plotted against both the Architect-Immediate (Figure) and the Roche-Stored results, the Architect-Stored results showed an average constant positive bias of 0.40 mg/dL. In contrast, the Architect-Immediate results and the Roche-Stored results correlated very well, with a minute bias of 0.01 mg/dL. To establish that a substance in the separator tubes was causing the erroneous increase of calcium in the Abbott assay, another group of 29 specimens was collected in Greiner Vacuette lithium-heparin tubes without separator (product number 454029). Again, these specimens were centrifuged and analyzed immediately on the Architect c8000. The specimens were then stored at 4°C in the original separator tubes for 7 days and reanalyzed with the Architect. The total calcium results by Architect-Stored showed a minimal negative bias of 0.05 g/dL when compared to results by Architect-Immediate (Figure). The bias, as described above, is present within 24 hours of storage with minimal subsequent increase. It is observed with various lots of Vacuette tubes and continues to be present at the time of submission of this article.

The Abbott Architect c8000 method of total calcium determination is based on the reaction of Arsenazo III dye with calcium in an acid solution to form a blue-purple complex that is measured at 660 nm. The Roche method is based on the reaction of cresolphthalein complexone in alkaline solution to form a purple complex that is measured at 2 wavelengths: 600 nm and 700 nm. Both methods are compatible with serum or heparinized plasma. The Architect

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The authors have no relevant financial interest in the products or companies described in this article.

Letters to the Editor

breast specimen may offer direct correlation with magnetic resonance imaging (MRI) coronal views of the breast, at the present time, most breast excisions in the United States follow mammography- or ultrasound-detected abnormalities, resulting in stereotactic-guided or ultrasound-guided excisions. Magnetic resonance imaging-guided excisions are still a minority. Moreover, MRI image evaluations vary between institutions and are dependent on the type of the magnet. Most images are read primarily in the axial or sagittal planes, and therefore would not correlate with the coronal plane of sampling.

Second, examination of breast tissue by large sections requires prolonged fixation times (resulting in lengthening the time for the case to be signed out), specialized laboratory equipment, and additional expertise that may not be easily available in a routine laboratory setting. Transportation of giant sections for consultation and storage would require specialized laboratory facilities. Specialized laboratory personnel and storage would require special handling and storage facilities. Immunostaining for diagnosis and/or biomarker evaluation would be difficult, if not impossible, in automated stainers, compounded by the impact of prolonged fixation on biomarker expression.

Our method of breast excision examination (serial sectioning along the long axis) allows for measurement of the ductal carcinoma in situ (DCIS) lesion in a 3-dimensional axis, with the largest dimension being recorded as the DCIS size. Concurrent x-ray of the slices allows correlation with mammographic calcifications and directed sectioning in large excisions. Because tissue slices are numbered, mapping of DCIS is possible, as is going back to the main specimen for additional sampling, should the need arise. All specimens are anatomically oriented by the surgeon; thus, margins are easily evaluated. Sectioning along the long axis creates smaller sections that are easily accommodated in tissue blocks. Evaluation of the complete margin is also accomplished easily. Our method does not compromise patient care, allows for a standardized method for evaluation of breast excisions in a routine laboratory setting, and provides an accurate measurement of DCIS size and evaluation of margins.
Letters to the Editor

The Value of Transcapsular Invasion in Patients With Thymoma

To the Editor.—We read with interest the manuscript from R. Gupta and colleagues, entitled “Evidence-based pathology and the pathologic evaluation of thymomas: transcapsular invasion is not a significant prognostic feature.”1 This is a meta-analysis leading to the conclusion that there are no significant prognostic differences between patients with stage I and patients with stage II disease; thus, transcapsular invasion should be of no clinical value in tumors lacking invasion of the neighboring organs or the pleura. However, this analysis does not take into account tumor histology. Most of the work done in the past 2 decades has been devoted to define new morphologic criteria able to help in assessing prognosis and providing adequate treatment. This was done because Masaoka staging alone can miss the true oncologic implications of thymoma; in fact, recurrence may appear in a fairly great percentage of patients with early stage thymoma. Nevertheless, these tumors, at least in early stages, have been repeatedly advocated as being benign: up to 35% were reported as benign at 5 years in the series reported by Blumberg and colleagues.2 The Marino and Müller-Hermelink3 and the World Health Organization (WHO)4 classifications greatly helped in this direction: there are histologic subtypes that do worse than others do. This newly developed classification is still under evaluation, but there are clearly at least 3 categories that behave differently: A/AB/B1, B2, and B35; type C is already kept separate. In the review from Gupta et al,1 the mean disease-free survival for stage I and stage II thymoma was 96.6% and 90.2%, respectively; there were 24 recurrences (1.7%) at stage I and 64 (6.7%) at stage II. We understand that these values did not show any significant difference; however, they show that there are differences between these 2 stages. Stating that they could be included in the same group might be misleading because there are no studies performing power analysis to exclude the possibility of errors because of small sample sizes and the effect of histology on recurrence. In fact, it could be assumed that these 2 stages should be treated in the same way, independent of WHO classification.

We are reviewing our series of stage II tumors. Until 1989, we have treated them without a standard approach: Some patients received adjuvant radiotherapy, and some did not. We had recurrences (18%) only in the group with either stage IIB type B or stage IIB type C tumors; most of those recurrences were outside the mediastinum. For this reason, we

[Image 40x489 to 363x728]

Bias plots for total calcium (mg/dL) for Architect 2-day-old specimens in gel separator tubes (blue diamonds) and Architect 7-day-old specimens in tubes with no separator (pink squares) versus Architect-Immediate.
have started to treat type B and C stage IIB tumors with adjuvant chemotherapy and radiotherapy. With this approach, there was no recurrence in the type B group, and there was a significant decrease of this complication in the type C group.

Because all the articles included in this meta-analysis used the WHO histologic classification (this was listed among the criteria for inclusion in the study), could the authors provide any evidence on the effect of histology on recurrence rates, especially in the stage II group? Do they agree with our words of caution about considering the Masaoka staging system as the only predictive variable?

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The authors have no relevant financial interest in the products or companies described in this article.

Dr Richard Bright—Father of Medical Renal Disease

To the Editor.—In the special section on renal pathology in the February 2009 issue of the Archives of Pathology & Laboratory Medicine appeared a very meritorious coverage of the topic, although I will admit medical renal disease is not one of my areas of interest. As the reader will know, our knowledge of medical renal disease dates back to the seminal contributions of Dr Richard Bright and I thought those interested in history might enjoy a few brief remarks about someone who was truly not only a great physician but also, from all we can glean these many years later, a wonderful person. Dr Bright was featured in the "Portrait in History" series of the ARCHIVES in 2000,1 and I encourage interested readers to look at that good review of his career, which supplements the comments made here.

Dr Bright is of course known as one of the "great men of Guy’s," along with Dr Thomas Hodgkin and Dr Thomas Addison. I think it fair comment that, sadly, Dr Bright's name is not as well known as the names of those other legendary figures if only, in significant part, because the eponyms honoring their contributions have fortunately remained, whereas the designation "Bright's disease" has faded from most medical texts because of the tendency of many contemporary writers to consider eponyms negatively, an opinion which I regret inasmuch as eponyms honor many greats of medicine.

Dr Bright was born into more prosperous circumstances than either Dr Hodgkin or Dr Addison, but certainly he repaid to the world any of his good fortune in being born into a family of significant means. Dr Bright and his immediate ancestors were from Bristol in the southwest of England, a particularly prosperous city at that time, in part because of the seafaring tradition and the exploration of North America and other parts of the world that was taking place then. Dr Bright was an exceedingly well-rounded person with interests in languages, mathematics, botany, and geology among others. In 1810, when he was a young man of 21, he went on an expedition to Iceland and conducted significant geologic studies as part of that expedition. Later, he traveled extensively in Europe and his book entitled "Travels From Vienna, Through Lower Hungary, With Some Remarks on the State of Vienna During Congress in the Year 1814" was published in 1818 and was considered an outstanding book of its type. It may be purchased today, but unfortunately, at great cost as I have found out! A more modest investment will obtain, for anyone interested, an excellent biography of Dr Bright by a descendant, Pamela Bright, which was published by Bodley Head in 1983 and entitled "Dr. Richard Bright." Dr Bright was appointed full physician to Guy’s Hospital (London, England) in 1824 and it was during the following years that his major work on medical renal disease was carried out. His observations were first reported in 1827. Dr Bright was a great physician and the demands of patient care ultimately affected his opportunity to do original work. Although a clinician, like many great physicians of that era—and, for that matter, in subsequent times—he had a major interest in pathology as this quote from the biography about him indicates: "[P]athology was to absorb him completely. Every organ that was put before him he examined and investigated in meticulous detail."2 I hope these brief remarks may stimulate some to learn more about this wonderful investigator, and certainly I hope it causes us all to reflect on his remarkable career and what he brought to our knowledge of disease, primarily renal disease, but even to some other areas.

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