

# Technology Insight: will systems pathology replace the pathologist?

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## SUMMARY

By using systems pathology, it might be possible to provide a predictive, personalized therapeutic recommendation for patients with prostate cancer. Systems pathology integrates quantitative data and information from many sources to generate a reliable prediction of the expected natural course of the disease and response to different therapeutic options. In other words, through the integration of relatively large data sets and the use of knowledge engineering, systems pathology aims at predicting the future behavior of tumors and their interaction with the host. In this Review, we introduce the methods used in systems pathology and summarize a recent study providing the first evidence of a concept for this strategy. The results show that systems pathology can provide a personalized prediction of the risk of recurrence after prostatectomy for cancer.

**KEYWORDS** pathology automation, predictive medicine, prostate cancer, systems pathology

## REVIEW CRITERIA

Rather than conducting a traditional literature search for relevant papers, the authors referred to their extensive personal collections of relevant publications. The references were selected in order to provide a guide to the quantitative tools used in systems pathology and to describe the emerging applications of this new approach to prostate cancer.

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## INTRODUCTION

Pathology is a bridging discipline that involves both basic and clinical biomedical sciences. In this context, it includes both descriptive and mechanistic approaches, with the final goals of further understanding the anatomic and functional changes and underlying molecular events involved in disease-related processes. Surgical pathology studies mainly comprise macroscopic and microscopic examinations, and involve the visual recognition of different patterns in cells and tissues. Through years of training and subspecialization, the pathologist develops a 'mental database' of patterns associated with diseases. The clinical utility of these visual analyses is remarkable, but is undermined by its subjectivity, lack of quantitation, and the difficulty in reliably correlating microanatomic findings with a patient's outcome. Systems pathology is an approach that can be used to overcome these limitations by integrating data from tumor-tissue architecture with the spatial distribution of molecular function and clinical information. The increasing role that systems pathology plays in the analysis of prostate cancer is the main focus of this Review.

The tools integrated under the rubric of systems pathology have one characteristic in common: they yield quantitative measurements that can be assessed by algorithms. They differ in a fundamental way from conventional histopathology, even if a central tenet of systems pathology is to extract information preserving the architectural spatial patterns and relationships of the tissues and cells. The hypothesis is that diverse, data-rich sets can be mapped on tissue structures, yielding information that can go well beyond what pattern recognition, such as Gleason grade, can achieve.

In this Review, we elaborate on the analytic capabilities that can be brought to bear on conventional histopathologic samples, suggest the possibility of automating histologic diagnosis and grading of prostate cancer, and provide an example of how to augment the predictive, personalized power of tissue analysis through systems pathology.

## ROLE OF PATHOLOGY IN PROSTATE CANCER DIAGNOSIS AND PROGNOSIS

The incidence of prostate cancer has dramatically increased during the last three decades. The sharp increase can be directly linked to the use of PSA screening tests for prostate cancer. When an abnormal PSA level is detected as the result of such a screening test, a transrectal biopsy procedure, which will yield a representative sample of the prostate gland, is indicated. When a diagnosis of cancer is established on a pathologic basis, and staging indicates organ-limited disease, radical therapy is instituted.

As well as establishing a diagnosis, pathologists assign a Gleason grade, or Gleason score, that constitutes the best predictor of the behavior of the prostate tumor and its rate of progression. It is generally agreed that low-Gleason-grade tumors (scores 2–5) can be managed conservatively, whereas tumors with a score of 8–10 warrant more radical therapy. The intermediate-grade tumors (scores 6 and 7) make up the majority of organ-confined prostate tumors and exhibit heterogeneous clinical behavior.<sup>1,2</sup> It has, however, been reported that Gleason scoring is prone to high variability in both intrapathologist and interpathologist readings. The high number of prostate needle biopsies and the complexity of their interpretation have added pressure to pathologists' daily workload. To date, no significant technological advances have been reported or are available to alleviate this pressure. Moreover, there is an acute need for prognostic parameters that can serve as reliable predictors of the course of the disease, guiding the appropriate therapeutic choice between conservative and more radical treatments for prostate cancer.

An additional factor that motivates the discovery and application of novel prognostic parameters is the number of instances in which prostate cancer is being diagnosed at a very early stage. About 3% of biopsies showing minute carcinomas, and, in such cases, an increasing number of radical prostatectomies, contain only residual cancer of questionable clinical significance.<sup>3</sup> Circumstances such as these provide the rationale for the following question: can we create tools that will enable us to predict the course of disease in a specific patient with organ-limited prostate cancer and to choose the appropriate therapy? And will these new tools relegate the pathologist to a minor role?

## THE ROLE OF SYSTEMS PATHOLOGY

Systems pathology represents a novel, comprehensive, and integrated approach to personalized

medicine. It integrates heterogeneous sets of high-dimensional data derived from quantitative object-oriented image segmentation and classification of digitized histopathology with digitized local and global expression signals of multiplexed immunofluorescent markers. Systems pathology relates these databases to conventional clinical and pathologic information and, when pertinent, it also uses data obtained from genomic or proteomic analysis. In contrast to conventional clinical and pathologic technologies, many of the approaches used in systems pathology preserve and take into account tissue architecture for the analysis of the data gleaned from tissue samples.

A defining characteristic of systems pathology is its computational platform: the mathematical wrapper, which is based on neural networks and machine learning kernels; these enable the integration of large numbers of data points and hundreds of variables. After the computations are complete, the end result renders a user-friendly report providing an individualized and personalized prediction for the patient. In the case of prostate cancer, this can include predictions of the time to disease recurrence and of clinical failure after radical prostatectomy.

Progress in understanding the molecular biology of neoplasia has yielded a plethora of molecular entities that can be used as prognostic and predictive markers, or as therapeutic targets, in many types of tumor. A number of regulatory pathways are known to be disturbed in prostate cancer, including apoptotic mechanisms, the androgen-receptor pathway, signal transduction, cell-cycle checkpoints, cell adhesion, and angiogenesis. From a large number of studies focusing on prostate cancer, recently reviewed by Quinn *et al.*,<sup>4</sup> only a limited number of markers emerge as having the potential to help predict outcome or response to therapy in patients with prostate cancer.

## TECHNIQUES USED IN SYSTEMS PATHOLOGY

A combination of clinical information, conventional histopathology, quantitative tools to assess concentration and localization of relevant proteins, *in situ* assessment of transcriptional activity for certain genes, automated analysis of conventional histopathology images, and computational integration of the data with advanced statistical analysis and machine learning are used to explore the predictive power of systems pathology (see Box 1 for definitions of molecular pathology terms).

### Morphometric features of tissues

Morphometric and texture features of structures in prostatic tissue have been used to identify glands and stroma, and can be used to discriminate between normal and cancerous regions of the prostate gland with a diagnostic accuracy of 79%.<sup>5</sup> Spectral imaging enabled Roula *et al.*<sup>6</sup> to achieve a classification accuracy of 94%, using a complex set of classes that included identification of benign prostatic hyperplasia, prostatic intraepithelial neoplasia, and a limited number of cancer classes. Texture features and the area occupied by the nuclei and lumina were among the data used to achieve the reported level of accuracy.

Attempts have also been made to obtain a Gleason grade through machine vision, an approach that uses algorithms to analyze data extracted from images.<sup>7,8</sup> Extraction of statistical and structural features from spatial distribution of epithelial nuclei over the entire image area and the use of a neural network, such as the Gaussian classifier, allows separation of moderately and poorly differentiated samples with an accuracy of 77%.<sup>9</sup> The power spectrum has also been used to represent the texture characteristics of tissue images, and the data analyzed by principal-component analytic methodology and nearest-neighbor classifiers (alternative statistical methods for data mining and analysis) had an accuracy of 90%.<sup>10</sup>

Other studies have used spanning trees connecting tumor cell nuclei to capture images of tumors with a preassigned Gleason grade. A 97% accuracy in the classification of images into tumor grades of between 2 and 5 was achieved by extracting features via co-occurrence matrices, wavelet packets and multiwavelets—all proven methods of data analysis and interpretation. The classification distance was optimized using simulated annealing. Finally, Arjam *et al.* used a classifier derived from shape and texture features. Morphometric and texture features from prostate glands are used in a series of classification stages to sort images into Gleason grades 1 to 5.

### Object-oriented image analysis

Object-oriented image analysis enables cell and tissue analysis to be performed in a manner similar to that of pathologists (Figure 1). Using the structural and relational information in the digital image to extract meaningful data has many advantages over traditional image analysis techniques. This approach requires an enhanced

**Box 1** Definitions of molecular pathology terms used.

#### Co-occurrence matrices

A matrix that describes the frequency of one gray tone coinciding with another gray tone in a specified spatial linear relationship within the area under investigation.

#### Gaussian classifier

In statistics, a classifier enables the mapping from a feature space (X) to a discrete set of labels (Y). The Gaussian classifier is a commonly used statistical classifier that assumes the data are normally distributed, and estimates the mean and the variance of the distribution.

#### Image object segmentation

A class of feature-extraction systems that uses images that can be semiautomated.

#### Multiplexed immunofluorescent markers

The simultaneous examination of a tissue sample using a variety of antibodies detected by immunofluorescence. The fluorochrome signals can be separated by spectroanalysis.

#### Nearest-neighbor classifiers

A biostatistical approach that assigns to a new object the class label of the most similar object in the previously classified database.

#### Power spectrum

The power spectrum is a fundamental characteristic of any signal, and is frequently used in engineering, electronics, communication, and related systems. It is a valuable tool in the analysis of the color characteristics of a signal.

#### Principal-component analytic methodology

A popular approach to filter out the noise in large data sets and reveal hidden structures present in the data.

#### Spanning trees

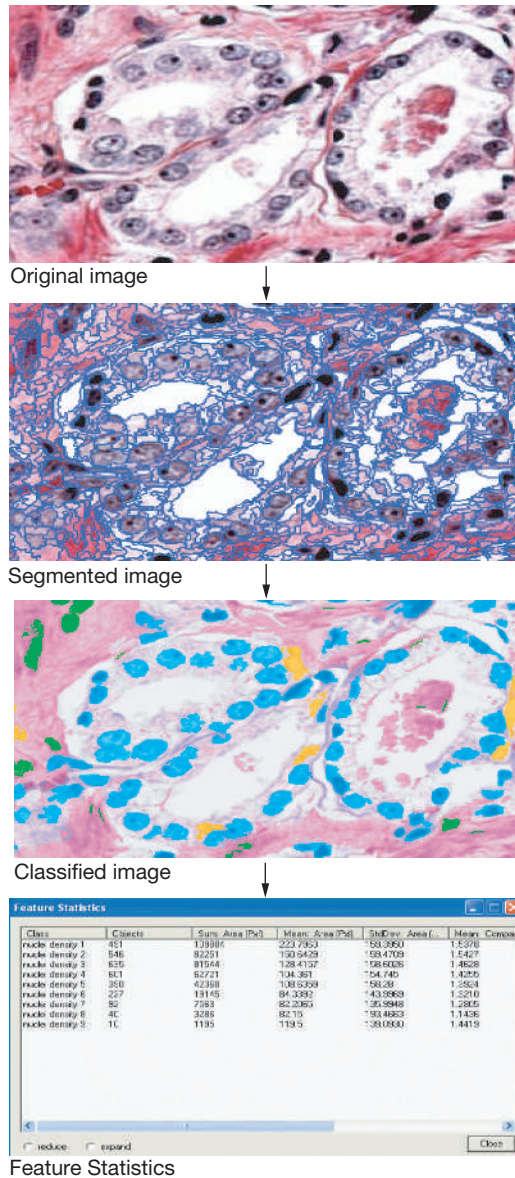
The minimum set of edges that connects all vertices of a graph.

#### Wavelet packets and multi-wavelets

The term 'wavelet' refers to the wave-like appearance of data as represented in a graph. Analysis of wavelets, wavelet packets (a collection of wavelet bases), and multiwavelets has led to the development of new signal-processing algorithms. Wavelet transforms have widespread applications in biomedical research, and the more evolved algorithms, such as wavelet packets and multiwavelets, have found uses in image-processing, blood-pressure, and heart-rate analysis, as well as in DNA and protein analysis.

quality of object-oriented image analysis, overcoming the limitations of pixel-based technology, which fails when the regions of interest in an image lack sufficient contrast to separate them from their surroundings. Object-oriented image analysis reduces the incidence of noisy and low-contrast image data that can severely limit the utility of the application.

Image object segmentation (IOS) technology can expand the traditional image analysis approach by adding the concept of a knowledge base. In IOS, image primitives like points, lines and regions are generated from the images. These primitives have user-defined attributes



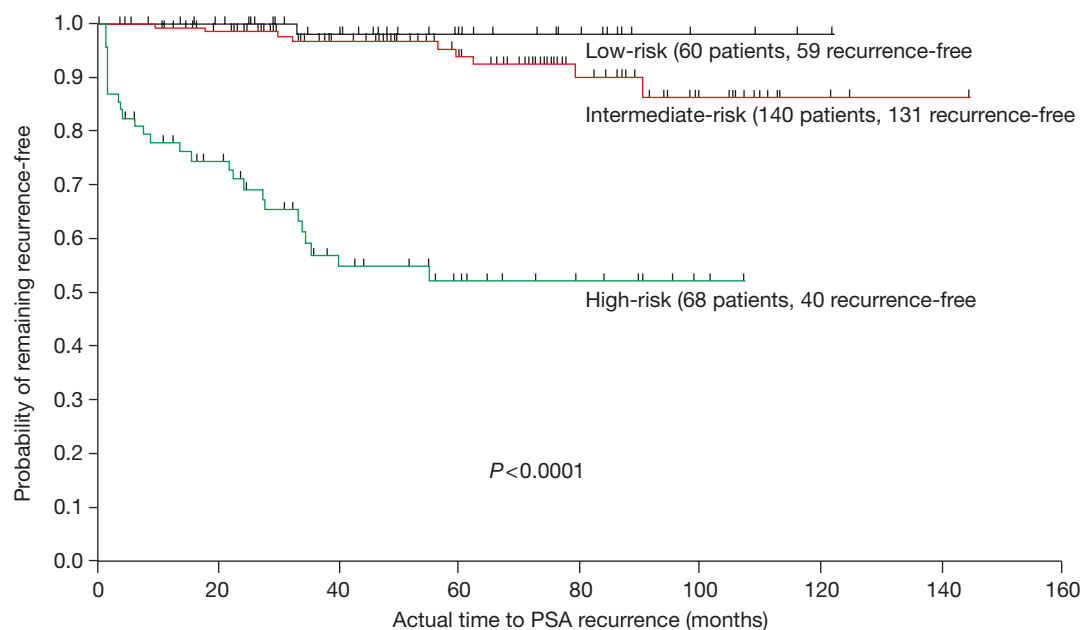
**Figure 1** The different steps involved in object-oriented image analysis. Hematoxylin and eosin images are used for image analysis and segmentation. For tissue segmentation, image objects are classified into histopathology classes from spectral and shape characteristics and spatial relationships between tissue objects. For the given histopathology object in this example, its properties were computed and output as morphometric features. Properties included both spectral properties and generic shape properties. Statistics were computed for each property specific to the histopathology object. Quantitative immunofluorescence was conducted in deparaffinized tissue samples. Images were taken with specialized camera equipment and image stacks were ‘unmixed’ using analysis software (from Cambridge Research & Instrumentation Inc., Woburn, MA). Typical regions of autofluorescence and other fluorescent objects were assigned to spectral profiles in order to complete the spectral library. After completion of the unmixing process, quantitative gray-scale TIFF images were stored for the analysis. Analytical and statistical results were generated by support vector feature reduction algorithms. All the features in the model were ranked in order of the absolute value of their contribution. When analyzing the feature statistics, the feature with the lowest contribution to the model is dropped and a new model is constructed on the remaining features. This procedure is repeated until there are no more features left for an algorithm model to be trained on. At this point, the model with the highest fitness is selected. In the case of multiple models with equal values of the fitness, the model with the fewest features is selected.

derived from their shape and spectral properties (Figure 1). External knowledge, such as the pathologist’s expertise of how the objects in the image should be mapped to histopathologic features, can be added to the knowledge base as well. The topology of the primitives is represented as a graph, where each vertex of the graph represents an image primitive, and the edges of the graph represent relationships between the primitives, like a neighborhood. This progressive assembly approach has many advantages over other methods (such as pixel-based and object-oriented methods) because it uses the image to generate the pattern-recognition capabilities by using supervised machine learning algorithms. The latter, as opposed to programmatic

approaches, are capable of accumulating experience that increases their efficacy.

**Algorithms and neural networks**

Supervised machine learning algorithms enable the training of the model, improving its ability to computationally classify a histologic pattern and establish its identity. Machine learning techniques are also becoming increasingly favored in the analysis of complex datasets that contain multiple sources of information, such as those generated by systems pathology. Statistical learning, a branch of modern machine learning proposed and developed by Vladimir Vapnik over the last two decades, opens a new avenue for solving problems with a small number of



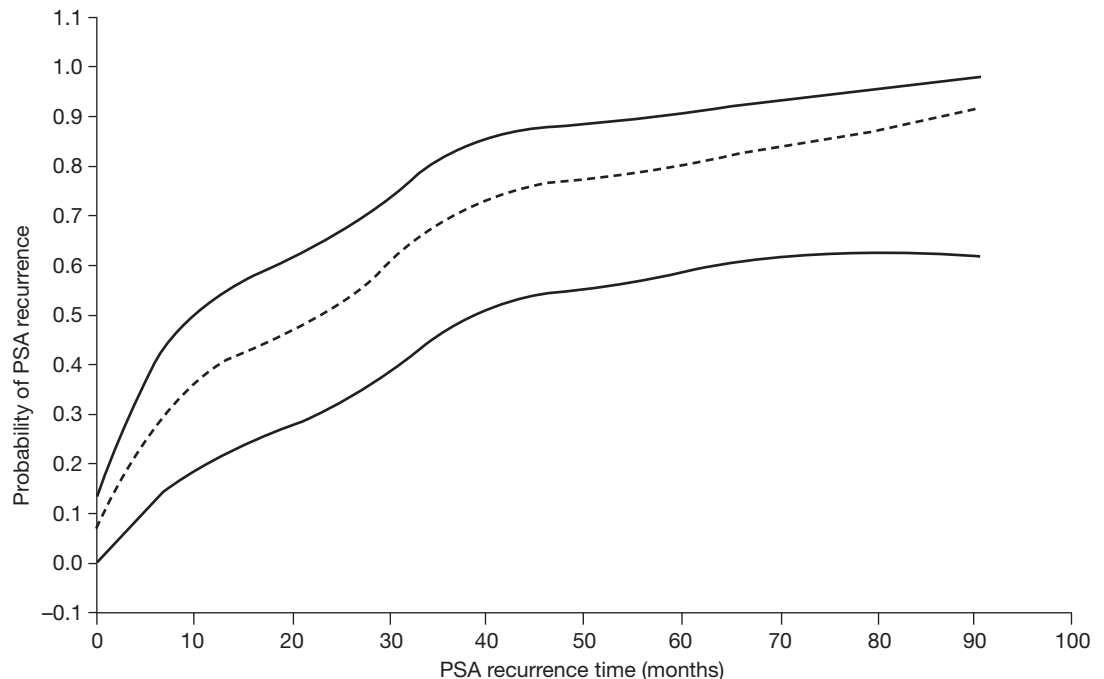
**Figure 2** Risk groups for PSA recurrence using the SVRc model score, illustrated as a Kaplan–Meier curve. This graph demonstrates the classification of patients as being at a low risk (<25%; black line), intermediate risk (25–75%; red line), or high risk (>75%; green line) of experiencing clinical failure. The probability of remaining free from clinical failure is provided by the y-axis, and follow-up time for PSA recurrence (in months) is given by the x-axis. Tick marks indicate censored patients. The *P* value is estimated using the log-rank test. Abbreviation: SVRc, support-vector-regression methods for censored data.

records in a large feature space; in other words, predicting the future trajectories from a small set of data points.<sup>11</sup>

The support vector machine (SVM) algorithm has quickly gained popularity in many applications because of its solid theoretical foundation, stable numerical computation, and generalization properties. One of the useful properties of SVMs is their capacity to solve problems with sparse and noisy data. This explains why it has become the method of choice to solve many problems in the fields of bioinformatics, text analysis, and handwriting recognition. SVMs have also been applied successfully to tumor classification.<sup>12,13</sup> They are capable of handling situations where the number of variables might be much larger than the number of samples, a situation known as the ‘curse of dimensionality’. In the area of DNA microarrays, SVMs have performed as well or better than other statistical analysis systems.<sup>14–16</sup>

An alternative machine learning strategy is the use of neural networks. Neural networks can be applied to survival analysis so that all the potential predicting factors can be evaluated simultaneously. For neural networks, there

have been two main approaches to handling censored patient data. The first approach models the hazard or survival function as an actual neural network structure. Brown *et al.* constructed the survival curve by a hazard function modeled using a neural network, for which the *i*th output is the estimated hazard within a time interval, *i*.<sup>22</sup> Biganzoli *et al.* used the time interval as an additional input to a neural network to model the survival probability<sup>17</sup> of a cohort of cancer patients. In a different approach, other authors used several separately ‘trained’ networks, each used to model the hazard function at different time intervals.<sup>18</sup> Although neural networks have been shown to outperform traditional statistical models, probably because of their capacity to model nonlinearities,<sup>19</sup> their application is limited because they require a large number of samples in the ‘training set’.<sup>20</sup> To help remedy some of these limitations, support-vector-regression methods for censored data (known as the SVRc model) and neural networks optimized on the concordance index have been developed at Aureon Laboratories to more effectively handle censored data.



**Figure 3** Risk of PSA recurrence for a specific patient. The model generated using training and validation sets of data output (employing machine learning techniques) can be applied to individual patients to generate a personalized risk curve for disease recurrence. The probability of PSA recurrence in a man with prostate cancer is provided by the y-axis, and time until PSA recurrence (in months) is given by the x-axis. The broken line indicates the probability of PSA recurrence over time in a man with prostate cancer. The solid lines indicate the 95% CI of the prediction generated by the model.

#### APPLICATION OF SYSTEMS PATHOLOGY

To test the predictive power of the systems-pathology platform, we carried out a retrospective study of 268 patients who had undergone radical prostatectomy for localized prostate cancer at the Baylor College of Medicine. These patients had a median of 5 years of follow-up without experiencing biochemical, or PSA, recurrence. Tissue microarrays were created with triplicate tumor cores taken from the prostate cancers of these patients.

Digital images of hematoxylin and eosin stained slides were captured using a SPOT Insight QE Color Digital Camera (KAI2000)<sup>TM</sup> (Diagnostic Instruments, Inc., Sterling Heights, MI) at  $\times 20$  magnification (24 bits/pixel images of  $1,600 \times 1,200$  pixels in a TIFF format). Immunohistochemical studies were performed in serial sections from the tissue microarrays, using antibodies to 12 biomarkers relevant to prostate cancer. These were quantitated by image analysis, and the information obtained was integrated with clinicopathologic data from each patient. Clinicopathologic variables, including age, preoperative PSA level,

tumor-node-metastasis stage, biopsy and post-operative Gleason grade, lymph-node and seminal-vesicle involvement, were considered as potential predictive features. Markers measured using immunohistochemistry included Ki67, CK18, CD45, CD68, CD34, androgen receptor, CK14, Cyclin D1, PSA, prostate-specific-membrane antigen, p27, and HER-2/Neu. The study end point was biochemical recurrence, defined as biochemical detection of an abnormal level of PSA after surgery. The time from surgery to biochemical recurrence (in months) was known for each patient.

Over 400 features derived from clinicopathologic information, image analysis, and molecular markers were evaluated for each case using the SVRc model, and the predictive performance of the model was estimated using the concordance index, sensitivity, and specificity (defined as the ability to accurately detect early recurrences [ $\leq 5$  years] and late recurrences [ $> 5$  years], respectively). Scores generated by the SVRc model were also used to define risk-stratified groups of patients and explore differences in time to PSA recurrence (Figure 2).

Of the 400 features originally analyzed, the final model included a total of 10 features, which included clinicopathologic, imaging, and molecular markers. The final model had a concordance index of 0.87, with sensitivity of 0.80 and specificity of 0.83. Using the log-rank test, a significant difference in PSA recurrence corroborated the definition of the low-risk, intermediate-risk, and high-risk groups generated by the SVRc model ( $P < 0.0001$ ). Within this cohort of patients, systems pathology also provided individualized risk curves for specific patients for biochemical recurrence. Compared with the risk curves for the cohorts, the individualized curves provide valuable information about individual patients (Figure 3), and have the potential to contribute to follow-up plans and therapeutic stances.

## CONCLUSION

Systems pathology, as defined in this Review, is an approach that could lead to diagnostic advances, by integrating the technological developments made in various fields of medicine and science. Systems pathology aims to provide a personalized and predictive assessment of an individual patient's disease, in order to better guide diagnosis and management. Despite the advantages of systems pathology, the pathologist as a diagnostician will not be replaced. Rather, he or she will be using these kinds of integrative tools to improve the accuracy of diagnoses, and to tailor management to individual patients.

## KEY POINTS

- Systems pathology integrates quantitative data from diverse sources to render a personalized predictive and prognostic report
- Systems pathology combines conventional clinicopathologic information with quantitative morphology and molecular data
- Systems pathology uses modern machine learning techniques to mine and interpret the data sets that produce a predictive personalized report
- Prostate cancer has been chosen to provide the first proof that systems pathology can be used in patient care
- Initial results show that it is possible to use systems pathology to provide a personalized prediction of the risk of recurrence after prostatectomy in men with prostate cancer

## References

- 1 Albertsen PC *et al.* (1998) Competing risk analysis of men aged 55 to 74 years at diagnosis managed conservatively for clinically localized prostate cancer. *JAMA* **280**: 975–980
- 2 Bianco FJ Jr *et al.* (2003) Ten-year survival after radical prostatectomy: specimen Gleason score is the predictor in organ-confined prostate cancer. *Clin Prostate Cancer* **1**: 242–247
- 3 Allan RW *et al.* (2003) Correlation of minute (0.5 mm or less) focus of prostate adenocarcinoma on needle biopsy with radical prostatectomy specimen: role of prostate specific antigen density. *J Urol* **170**: 370–372
- 4 Quinn DI *et al.* (2005) Molecular markers of prostate cancer outcome. *European J Cancer* **41**: 858–887
- 5 Diamond J *et al.* (2004) The use of morphological characteristics and texture analysis in the identification of tissue composition in prostatic neoplasia. *Hum Pathol* **35**: 1121–1131
- 6 Roula MA *et al.* (2002) A multispectral computer vision system for automatic grading of prostatic neoplasia. In *Proceedings of the IEEE International Symposium on Biomedical Imaging*, July 7–10 2002, Washington, DC, 193–196
- 7 Stotzka R *et al.* (1995) A hybrid neural and statistical classifier system for histopathologic grading of prostate lesions. *Anal Quant Cytol Histol* **17**: 204–218
- 8 Smith Y *et al.* (1999) Similarity measurement method for the classification of architecturally differentiated images. *Comp Biomed Res* **32**: 1–12
- 9 Wetzel AW *et al.* (1999) Evaluation of prostate tumor grades by content-based image retrieval. In *27th AIPR Workshop: Advances in Computer-Assisted Recognition*, 14 October 1998, Washington, DC, **3584**: 244–252 (Ed. Merickso RJ)
- 10 Jafari-Khouzani K and Soltanian-Zadeh H (2003) Multiwavelet grading of pathological images of prostate. *IEEE Trans Biomed Eng* **50**: 697–704
- 11 Vapnik VN (1995) *The Nature of Statistical Learning Theory*. New York: Springer-Verlag
- 12 Su AI *et al.* (2001) Molecular classification of human carcinomas by use of gene expression signatures. *Cancer Res* **61**: 7388–7393
- 13 Yeang CH *et al.* (2001) Molecular classification of multiple tumor types. *Bioinformatics* **17** (Suppl 1): S316–S322
- 14 Cristianini N and Shawe-Taylor J. (2000) *An Introduction to Support Vector Machines and Other Kernel-Based Learning Methods*. Cambridge, UK: Cambridge University Press
- 15 Ye QH *et al.* (2003) Predicting hepatitis B virus-positive metastatic hepatocellular carcinomas using gene expression profiling and supervised machine learning. *Nat Med* **9**: 416–423
- 16 Brown MPS *et al.* (2000) Knowledgebased analysis of microarray gene expression data using support vector machines. *Proc Natl Acad Sci USA* **97**: 262–267
- 17 Biganzoli E *et al.* (1998) Feed forward neural networks for the analysis of censored survival data: a partial logistic regression approach. *Stat Med* **17**: 1169–1186
- 18 Ohno-Machado L and Musen MA. (1997) Modular neural networks for medical prognosis: quantifying the benefits of combining neural networks for survival prediction. *Connect Sci* **9**: 71–86
- 19 Kattan MW *et al.* (1998) Experiments to determine whether recursive partitioning or an artificial neural network overcomes theoretical limitation of cox proportional hazards regression. *Comput Biomed Res* **31**: 363–373
- 20 Brown SF *et al.* (1997) On the use of artificial neural networks for the analysis of survival data. *IEEE Trans Neural Netw* **8**: 1071–1077

## Competing interests

The authors have declared associations with the following company: Aureon Laboratories. See the article online for full details of the relationship.