The biobank resource in anatomical pathology

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Introduction

Activities in a pathology department are mainly concerned with supplying physicians with diagnoses of specimens from their patients. The sample material consists of tissues or cells. These specimens are prepared in a laboratory process whereby thin sections of tissue specimens or cell smears are placed on slides and then stained, so that details of tissues and cells can be studied in the microscope. The pathologist, however, makes his first observations of the incoming material with the naked eye, before attempting, through the microscope, with the background of his experience of samples examined previously and with reference to given diagnostic criteria, to interpret any lesions that may be present in the material received. Examination of the specimen material should normally lead to a statement describing the findings in the material and leading up to a diagnosis or to various possible differential diagnostic options. Although microscopic scrutiny as a form of image interpretation must also be conducted as objectively as possible, that scrutiny nevertheless involves elements of subjectivity. The difficulty of assessment varies from one specimen to another. In some cases diagnosis comes easily and reproducibility is high between different diagnosticians. In others the findings are so hard to interpret that different diagnosticians can arrive at different assessments of the specimen material. If specimens are this hard to interpret, diagnosticians with special experience of a certain type of specimen material or of certain diagnoses are often consulted.

Clinical pathology and cytology

Clinical pathology and cytology span a wide diversity of pathological states, so much so that diagnosticians in the larger pathology departments have had to sub-specialise. Thus we now have specialists, for example, in breast pathology, dermato pathology, endocrine pathology, gynaecological pathology, haemato pathology, lung pathology, neuropathology, urological pathology and gastrointestinal pathology. To optimise their diagnostic service to hospital departments sending material for analysis, pathology specialists with specialist knowledge also take part in recurrent clinico-pathological conferences together with clinicians. At these conferences the pathologist demonstrates specimens for microscopy and diagnosis, differential diagnoses and the subsequent course of examination and treatment are discussed, often resulting in a consensus decision.

Technically speaking, the handling of specimens in the laboratory is very much of a handicraft and is based on the fundamental principle that incoming tissue specimens first have to be fixed so as to curtail all processes of degradation in the tissue. This is followed by a dehydration process which removes water and fixer from the specimen, substituting a paraffin solvent. The tissue material is then embedded in paraffin, after which the specimen will be preserved in a paraffin block. From this block, thin sections of the tissue are then cut and placed on glass slides. With the tissue section now in position on the slide, the next stages of preparation are deparaffination and staining. The purpose of the staining procedure is to make various details of the tissue distinguishable to the eye, so that cells, cell nuclei and other tissue structures will be made visible in the microscope.

The diagnostic process is basically microscopic, i.e. based on *examination in the microscope* and on an assessment of findings in the specimen material with reference to diagnostic criteria for different diseases. A conclusive diagnosis, however, cannot always be reached solely through microscopy of the specimen material. New techniques have also made it possible for other kinds of information in the tissue material to be revealed. One technique which has rapidly gained ground is *immunohistochemistry*, which means that, by using antibodies, e.g. for various proteins, one can visualise whether or not these proteins are present in a tissue specimen.

Immunohistochemical stainings, then, mean that antibodies to various structures in the tissue are incubated with tissue sections mounted on slides and any antibodies attached to structures in the tissue are then “developed” in a chemical reaction which makes the antibody and its location in the tissue visible through the microscope. For example, in a case of undifferentiated malignant tumour – where microscopy does not enable us to judge where the tumour emanates from – the demonstration of cytokeratine in the tumour cells, by means of immunohistochemical technique, can show the tumour to be of epithelial origin. If instead an undifferentiated malignant tumour of this kind expresses leukocyte common antigen, this means that the tumour emanates from lymphocytes instead.

The panel of antibodies available for these purposes today exceeds more than a hundred, and this possibility of visualising structures not observable with the naked eye has made microscopic diagnosis more certain, with the result that more specific diagnoses can now often be made. In certain special diagnostic situations, *molecular genetic techniques* can also be used for extracting further information from a tissue specimen. In connection, for example, with cervical cell changes, a special examination technique can establish the presence or absence in the specimen of human papilloma virus. Any such virus, if present, can be typed, and in this way it can be ascertained whether the patient is infected with “dangerous” or “harmless” variants of the virus. In addition, for example in cases of infantile leukaemia, these molecular genetic techniques can tell us whether treatment of the leukaemia has eliminated the leukaemia cells from the bone marrow or whether they are still present.

The tissue specimens sent to the pathology department include a certain proportion of surgical specimens, i.e. entire organs or parts of organs which have been removed for purposes of treatment or diagnosis. Material of this kind can, for example, take the form of a inflamed gallbladder, a spleen removed because it is destroying the patients blood platelets, part of an extremity with a malignant tumour, or tonsils which have become enlarged, causing the patient difficulty in breathing or swallowing. Tissue specimens can also take the from of biopsies, i.e. small samples of organs or tissues where the physician treating the patient suspects a pathological process. This may be a suspected malignant tumour which has to be diagnosed in advance of surgery or some other kind of treatment, in order for the right treatment to be started. The material can also come from curettage, e.g. of the womb, in which case the physician treating the patient performs curettage of the endometrium with a view to diagnosis or as treatment for a haemorrhaging condition. Needle biopsy is a relatively new technique whereby samples can be removed from a sick tissue. This minimal material is sometimes all that is needed for a complete diagnosis. Cell specimens consist of cells scraped, for example, from the cervix, cells isolated from fluid effusions in the thoracic or abdominal cavity, or cells “aspirated” from tissue or organs by a fine needle biopsy technique.

The tissue or cell specimen is accompanied by a referral from the physician treating the patient, supplying anamnestic information and a description of the patient’s symptoms and the clinical findings. The referral information also includes a question which the physician requires an answer to. The information in the referral is often supplemented by a certain amount of laboratory data, as well as particulars of earlier samples examined from the same patient and the diagnoses obtained.

After a diagnosis has been sent to the physician, the sample material, in the case of tissue specimens, consists of stained sections from the specimen mounted on slides, as well as blocks of paraffin wax in which the sections on the slide were previously embedded. The preparations made from incoming cell specimens consist solely of stained cells on slides. The sample material left over after the specimen has been embedded and slides prepared is retained for about a month and then destroyed as biological waste. During the month for which the whole of the macroscopic sampling material is stored in the pathology department, the diagnostician is free to return to the material and, if the examination so requires, to remove additional sections for embedding and microscopic examination. This sometimes becomes necessary if the first sample of pieces examined does not yield a diagnosis or if relevant portions of the diseased parts have not been included in the pieces selected. Sometimes access to the macroscopic preparation is also needed to facilitate the investigation of a mix up of specimens, in which case the physician sending in the specimen may need to be contacted for further information about the character of the material sent in.

Reasons for preserving specimen material

The General Recommendations issued by the National Board of Health and Welfare[1] advise pathology departments for the time being to retain paraffin wax blocks and slides with cytology specimens together with the referrals accompanying sample material of all kinds. The National Board of Health and Welfare recommends at least 15 years’ retention of slides prepared from the embedded material and cell smears from the cervix. There are several reasons for these retention routines. One important reason is concerned with *care of the patient* and the investigation of the illness which the patient may be suffering from. In the event of a relapse one needs to go back to the first samples that were sent in, in order, e.g. when investigating a tumour, to compare the latest specimen received with an earlier one. In this kind of examination it is important to decide whether the patient has suffered a recurrence of a tumour diagnosed previously or has developed a new tumour. Clarification of these matters has an important bearing on the planning of treatment and on the individual patient’s prognosis. In such an investigation it may be necessary to carry out additional examinations of the old sample material in order to refine the diagnosis of that material, e.g. if new and more advanced techniques of diagnosis have been developed in the meantime.

The preserved material is also an important resource for all other *medically related activity* in a pathology department. The embedded material is used to a certain extent in development work on the laboratory process and for testing new methods whereby more information can be derived from the sample material. The immunohistochemical stainings mentioned earlier resulted from comprehensive development work using paraffin blocks. This material will also need to be used in the future as a means of further optimising the technique, so that different structures in the tissue can be visualised in the best possible way in sections from embedded samples. This means that different types of tissue will have to be used in order for different pre-treatments of the material to be evaluated, so as to make the structures which are to be demonstrated by means of antibodies accessible to those antibodies. Once an immunohistochemical staining technique has been tried out, whenever the technique is used a positive control has to be used on the same slide as a specimen from a patient, so as to make sure on this particular occasion that the staining works. To this end, embedded material with the positive control has to be all the time available for use.

New antibodies are appearing all the time, making it possible for new structures to be demonstrated in tissue and cell specimens, and this development work, accordingly, is continuous and demands access to a comprehensive material of paraffin wax blocks. Similarly, increasing use is now being made of molecular genetic techniques to supplement the microscopic assessment of the sample material. In accordance with this it is becoming increasingly common for *in situ* hybridisation to be used on routine material. With techniques of this kind, chromosomal injuries of different kinds can also be demonstrated in paraffin wax sections or cell specimens by incubating DNA specimens with a connected fluorescent substance in the tissue sections. These DNA specimens identify specific parts of chromosomes, and in this way "translocations" between chromosomes or "amplifications" of parts of chromosomes can be demonstrated. If a technique of this kind is put into service, comprehensive development work is needed to make it viable. This technique also demands constant access to control material, so that one can be sure of the technique having worked properly whenever it is used.

Who uses the sample material?

The material in a pathology department's biobank is also used in the training of prospective pathologist and cytologists and for developing the specialists' competence. For this purpose the embedded material may sometimes need to be re-used, by making new sections and preparing different stainings. Slide seminars are a frequently recurrent form of instruction in pathology. They involve producing several editions of a collection of preparations, e.g. from tumours of the lymphatic glands, for distribution to all pathology departments in the country or to the participants on a course. In such slide seminars, pathology departments are often invited to submit suggested diagnoses of the cases included in the seminar box, and at a meeting of specialists from the pathology departments an expert in the field reviews the cases concerned, shows examples of findings made in the preparations, demonstrates special staining techniques and discusses diagnosis and differential diagnoses. On courses, e.g. for residents during their specialist training, the teacher gives a very hands-on presentation of each individual preparation, discusses different findings and how to diagnose different preparations, gives advice on pitfalls and how to avoid them, and discusses various differential diagnoses. This type of training and instruction is always going on within the profession and is absolutely dependent on access to the specimen material held by the pathology departments.

Another medically related use of the sample material concerns occasions when new classification systems are published for different groups of tumours. In order for experience concerning these classification systems and their usefulness to be acquired swiftly, and to give people an opportunity of practising the use of them, it is often necessary to compile and review large corpuses of material from several patients with tumours coming under the new system of classification. New sections and stainings from the preserved material may then be needed. Retrospective reviews like this of collections of preparations are also made necessary when clinical treatment programmes come up for evaluation. On such occasions, the sample material from the patients whose treatment is to be evaluated has to be reviewed by an experienced pathologist on one and the same occasion. Before such a review can take place, new sections and stainings often have to be prepared in order for the sample material to be processed as uniformly as possible.

A further important reason for the retention of sample material and referrals at pathology departments, as remarked in the General Recommendations of the National Board of Health and Welfare, concerns the use of the material for *research*. This combined research resource of all Sweden's pathology departments has been estimated at between 40 and 80 million paraffin blocks. The material represents a host of different diseases with morphological expressions, including diseases which are already well known and those which have yet to be identified or fully described. In future, then, this material may provide answers to important questions about the nature of diseases, their origin and development. This type of research based on tissue and cell specimens has been going on for over a century now, and the material accumulated by the pathology departments has been a very great asset in the overall advancement of medical science. Previous studies based on this archived material have mostly been confined to morphological descriptions, both macro- and microscopic, of diseases. But, in addition to morphological details, which identify different diseases in many ways, the sample material also conceals other information which is tending more and more to become a subject of research. This type of research has been made possible by new techniques which in various ways have been capable of revealing hidden information in the tissue material, above all by indicating different proteins and other components in cells and tissues which cannot be made visible by traditional staining techniques. Immunohisto-chemical stainings use antibodies to various proteins and other structures in cells and tissues. By incubating sections with antibodies marked with a stainable substance, the presence of such proteins can be demonstrated in the sections and their location in the sample material described. In this way, changes in the diseased tissue can be described more closely in relation, for example, to the behaviour of other tumours under consideration, and also in relation to the way in which normal tissue expresses the protein investigated. This type of research has yielded many new results which have simplified and refined morphologically based diagnosis, making groups of tumours, for example, more easily distinguishable from each other and for tumours with an undifferentiated morphological expression to be traced back to their origin. Other techniques, e.g. chemical methods, may also be applicable to the embedded material.

Conclusion

The introduction of molecular biological techniques has opened up a new dimension in the retrieval of information hidden in the preserved sample material. These techniques make it possible to reveal specific changes in the genetic material. Changes thus demonstrable include, for example, various kinds of chromosomal damage such as translocations, deletions and mutations. The presence of foreign DNA material – virus, for example – can also be demonstrated by these methods. In this way chromosomal changes predisposing for the occurrence of a certain kind of tumour or other diseases can be mapped and studied more closely. Thanks to the retention of large numbers of samples in various pathology departments, large bodies of material can be composed for particular diseases. One limiting factor in these molecular biological studies of the sample material has been that the DNA extracted from the paraffin wax blocks is inferior in quality to DNA isolated from fresh tissue. During processing of the tissues, the DNA is often affected in such a way that chromosomal fractures appear, impeding the use of the material. This is one of the reasons why the sample material sent to pathology departments often arrives nowadays in the fresh state and is partly deep-frozen for future studies of different kinds, both medically motivated and research-related.

It is impossible to predict the new techniques which research may come to employ in the future and the needs this will generate for sample material from the pathology departments. Clearly, though, the length of time for which sample material needs to be stored is infinite, i.e. future preservation will have to be for an indefinite term.

References:

[1] SOSFS 1995:9 (M)