Clinical review

Science, medicine, and the future

Molecular assessment of cancer

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The recent announcement of cancer as the main cause of death in the United Kingdom comes at the end of two decades of exciting advances in understanding the basic biology of human tumours. It is now established that the development of neoplasia is due to accumulated genetic alterations in somatic cells. Identifying cell populations that share specific genetic alterations from samples of saliva, sputum, urine, stool, and blood is likely to be become a routine method of screening populations for common cancers. Genetic assessment will also be used to stage tumours and detect micrometastases. The correlation of specific profiles of genetic alterations with clinical outcome will help define prognosis more accurately and enable clinicians to target treatment more effectively.

Possible clinical futures

- Detailed definition of genetic alterations and altered patterns of gene expression in all the major human cancers will soon be available
- Obtaining the genetic profile of a patient's primary tumour will become routine to define prognosis and optimise treatment
- Large scale, population based trials of cancer screening by means of molecular assays in clinical samples obtained non-invasively (saliva, sputum, urine, stool, mucosal washings) will be conducted
- Molecular analysis of surgical margins, lymph nodes, and blood will be part of routine cancer staging
- Molecular detection of early preinvasive and preneoplastic lesions may allow cancers to be cured before they become symptomatic

Cancer as a disease of genetic alterations

The major discovery in cancer biology has been that tumorigenesis is a multistep process associated with accumulated genetic alterations in somatic cells. The progression of a tumour through preneoplasia to frank neoplasia and then invasion and metastasis is the result of successive rounds of clonal expansion of somatic cells that acquire a selective growth advantage as a result of mutations in genes that control cellular proliferation and death.[1] Mutations result either in the activation of oncogenes, which promote cellular proliferation or inhibit cell death, or in the inactivation of tumour suppressor genes, which inhibit proliferation or promote cell death. To become a cancer cell, a normal cell needs to accumulate at least five or six of these mutations.[1]

Mutations occur at a higher rate in cancer cells because their genetic material (chromosomes or DNA) is intrinsically unstable.[1] This genetic instability seems to be a "property" of cancer cells.[1]

Colorectal carcinoma is the best studied example of the genetic alterations that underlie human cancer.[2] A normal mucosal cell with inactivation of a tumour suppressor gene called APC will proliferate and became a small adenomatous polyp. Mutations in oncogenes (for example, K-ras) and tumour suppressor genes (for example, P53 and DCC) will then occur and lead to the transformation of the polyp into a larger adenoma, from which a carcinoma can eventually arise. Throughout this progression colorectal cancers display genetic instability. In about 15% of colorectal cancers this results in subtle sequence alterations at simple repeats of DNA nucleotides (called microsatellite instability) due to a defect in the ability to repair mismatches during DNA replication.[2] In the other 85% of cases there are gross chromosomal alterations, probably due to defects in mitotic segregation or mitotic recombination, or both.[3] Although not as well defined, similar genetic alterations have been described in most human cancers.

Molecular markers of cancer: mutations and genetic instability

Since both mutations and genetic instability are responsible for the biological characteristics of tumour progression it is easy to appreciate why their detection in populations of cells might become a useful diagnostic tool and even be predictive of clinical outcome.

Genetic alterations can be detected either by analysing the DNA isolated from cells or their chromosomes. The ability to amplify DNA about a million times by the polymerase chain reaction allows the study of very small amounts of tissue (fig 1a). The DNA can then be analysed for mutations, deletions, or

microsatellite instability. To use a mutation as a molecular marker, however, requires not only knowledge of the nucleotide sequence of the gene but also of the specific alteration within the gene (fig 1b). Defining the changes in sequence within cancer genes is difficult and time consuming, which is a disadvantage for widespread clinical application.

Analysis of DNA amplified by the polymerase chain reaction can also be used to look at chromosome deletions (fig 1c), which tend to occur at the sites of tumour suppressor genes, and to detect microsatellite instability (fig 1d), which occurs in up to 20% of human cancers.[4] Microsatellite alterations in cancer cells, either deletions or instability, are useful markers because their analysis is simple, fast, and cheap, and the process can be easily automated.

Once a cancer-specific mutation is identified by sequencing of DNA isolated from the primary tumour, the mutation can be used as a marker to detect rare cancer cells in the middle of thousands of normal cells, either in pathological specimens or in clinical samples. Cytogenetics is an alternative way to detect genetic alterations in cancer cells by analysing their chromosomes. Modern techniques, particularly fluorescent in situ hybridisation, are relatively simple and reproducible and can be used to look at gross chromosome alterations like aneuploidy, translocations, and deletions.

Early diagnosis of cancer

The molecular methods described above are able to detect the presence of mutant genes originating from abnormal cells in small lesions or from small numbers of cancer cells shed into clinical samples. Because the tests are both sensitive and specific they hold enormous potential for early diagnosis and detection of recurrence of cancers.

In 1991 Sidransky et al detected mutations of the P53 tumour suppressor gene in urine samples from patients with bladder cancer that were identical to the mutations in the primary tumour (fig 2).[5] Sidransky's group showed that a P53 mutation could be detected in a urine sample collected nine years before the diagnosis of bladder cancer was made.[6] In a subsequent study microsatellite changes matching those in the tumour were detected in the urine sediment of 19 of 20 patients with a diagnosis of bladder cancer, whereas urine cytology detected cancer cells in only nine out of 18 of the samples.[7] These results show that molecular detection of bladder cancer is a more sensitive screening tool than urine cytology.

The same group identified mutations in the K-ras oncogene in the stools of patients with curable colorectal tumours.[8] The successful detection of mutations in K-ras and P53 genes in several clinical samples (urine, stool, pancreatic juice, blood, sputum, saliva), not only from patients with cancer but also from those who later develop cancer and people at increased risk of developing cancer, illustrates the potential of molecular methods in the early diagnosis of cancer.[9 10]

Large scale screening programmes to detect non-invasive preneoplastic lesions and early cancers

Microsatellites

- These are tandem repeats of nucleotides (usually the dinucleotide CA) that vary in number at a particular site among homologous chromosomes
- They are of unknown function and are randomly dispersed throughout the human genome
- Individuals are often heterozygous for the total length of the microsatellite sequence at a given locus, and this size difference can by analysed by gel electrophoresis (fig 1c and d)

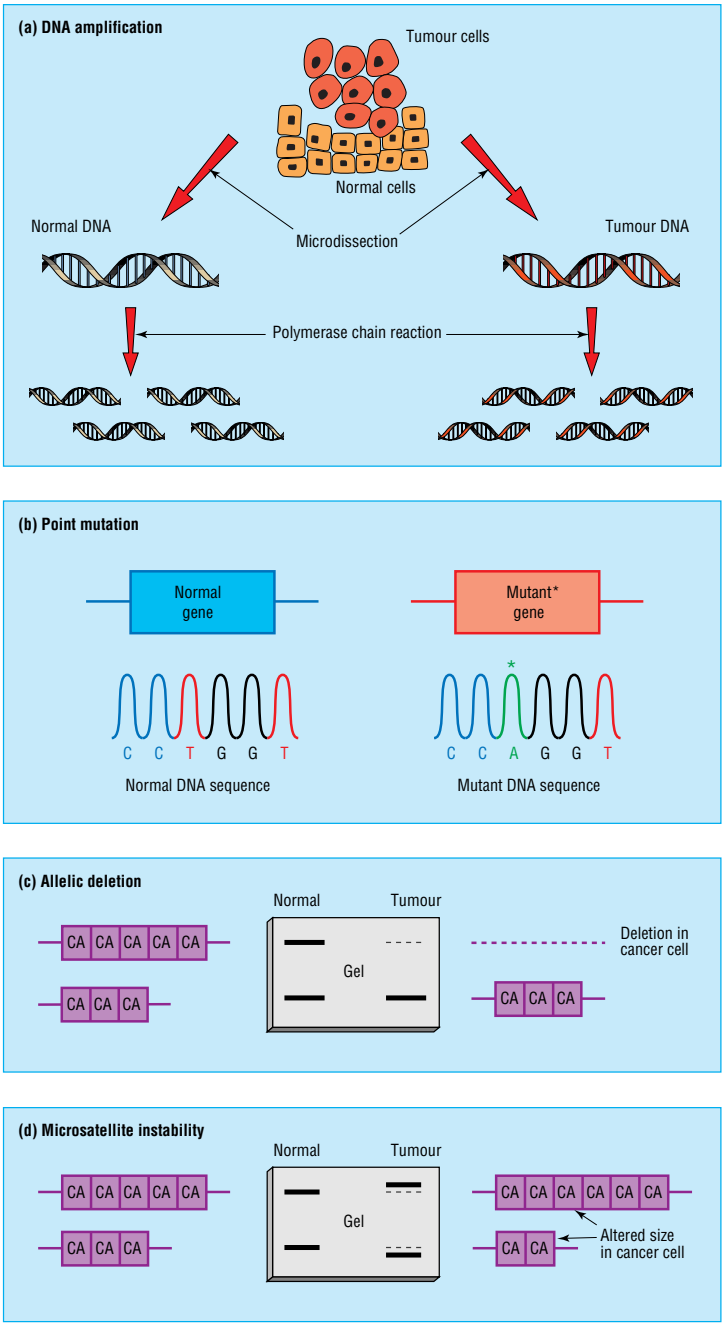

Fig 1 Detection of genetic alterations present in cancer cells. Microdissection is used to separate tumour cells and normal cells, from which DNA is isolated and amplified by polymerase chain reaction (a). If a specific gene is amplified, its DNA can be sequenced to reveal a point mutation (b). If anonymous DNA nucleotide tandem repeats (such as the microsatellite "CA") are amplified and analysed by gel electrophoresis, each band seen on the gel represents one of the two paired homologous chromosomes. If the cancer cell is missing one chromosome, one of the two bands is not seen in the DNA from tumour cells (c). If the cancer cell has a defect in mismatch repair (see text), the bands in the DNA from tumour cells are different in size from the bands in normal DNA (d)

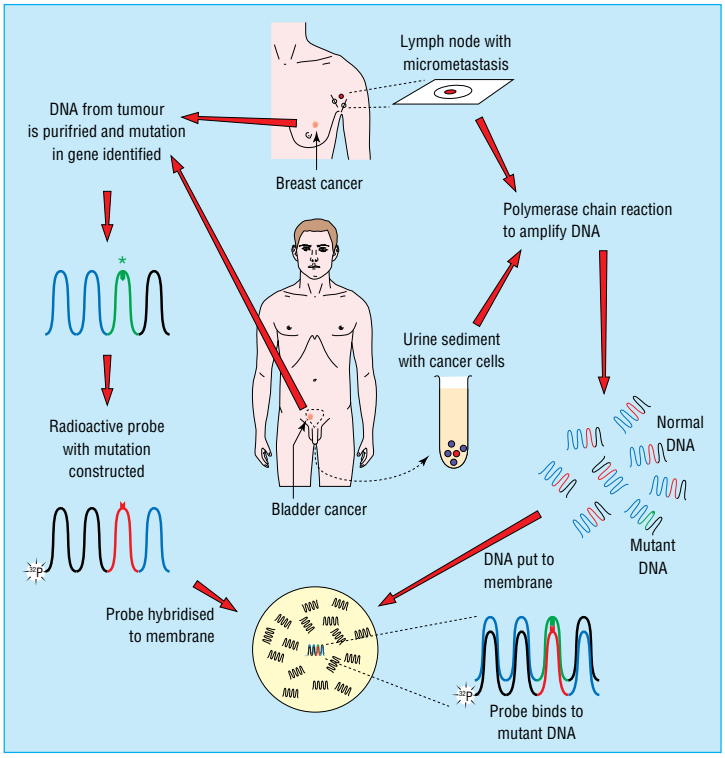

Fig 2 Detection of cancer-specific mutations in pathological specimens or clinical samples. DNA is purified from cancer cells and sequenced to identify a cancer-specific mutation. A radioactive probe containing the cancer-specific mutation is then constructed. DNA is isolated from axillary lymph nodes (for breast cancer) or from cells present in urine sediment (for bladder cancer) and amplified by polymerase chain reaction. Among the copies of DNA a small percentage will originate from cancer cells from micrometastases or shed into the urine. The copies of DNA are then put on to a membrane. The radiolabelled probe is hybridised to the membrane to detect the rare cells containing the mutation identical to the one present in the tumour

have been shown to be effective in reducing morbidity and mortality. This is true for both screening for cervical cancer and mammography for breast cancer.[11] However, overdiagnosis and falsely reassuring negative results are a major problem. The addition of molecular methods of cancer assessment—as an addition to cytological evaluation of cervical smears or to assay nipple aspirates from women at increased risk of breast cancer—could greatly reduce this problem.

Such tests would obviously have to show increased sensitivity and specificity in carefully conducted clinical trials. Such trials are already ongoing in the early diagnosis of primary or recurrent bladder cancer (using urine sediment), head and neck cancer (using saliva), and lung cancer (using sputum).

Molecular staging of human cancers

Correctly evaluating locoregional spread of cancer is of prognostic and therapeutic importance. Currently, assessment is based on histological study of surgical margins of tumours and regional lymph nodes. The problem with this is that small foci of metastatic cancer can be missed because of sampling problems or because cell morphology is often insufficiently informative. Molecular assessment of tumour margins and lymph nodes should improve the accuracy of staging. In addition, the ability to detect circulating cancer cells in peripheral blood samples may enable evaluation of micrometastases and tumour burden.[10]

In a recent study of 25 patients with head and neck cancer who were judged to have had a complete resection of their tumour on the basis of a negative histopathological evaluation, half had at least one tumour margin positive for a P53 mutation identical to that of the primary tumour.[12] These patients had significantly more local recurrences than those with no evidence of genetic mutation (5/13 *v* 0/12). Further trials are under way to determine the efficacy of molecular analysis in such patients.

Similarly, in patients with colorectal cancer it has been shown that genetic analysis to detect P53 or K-ras mutations in lymph nodes is a more sensitive indicator of metastatic spread than pathological analysis.[13] The potential clinical value of chromosome analysis in staging is illustrated by a report of single metastatic tumour cells detected in lymph node tissue from a patient with renal cell carcinoma.[14]

Molecular profiles to predict outcome and guide treatment

By identifying a cancer patient's genetic profile (which may, for example, indicate the likely sensitivity to radiotherapy) and the molecular profile of the cancer, and then correlating these with clinical outcome, we will eventually improve our ability to predict outcome and target treatment more effectively. Better prognostic markers are needed, especially for breast cancer—among patients whose axillary nodes are negative for disease, it is hard to define which patients need adjuvant chemotherapy.

The value of molecular markers was first suggested by a report that, among patients with breast cancer, increased relapse and decreased survival was associated with amplification of the HER-2/neu oncogene.[15] Subsequently, studies of a total of almost 1000 breast cancer patients have directly correlated P53 mutations in the primary tumour with prognosis. All have shown the mutations to be strongly associated with worse outcome, with a relative risk of recurrence between 2.2 and 4.7 and of death between 2.9 and 23.2.[16] If confirmed in large prospective clinical trials, these findings suggest that patients with P53 mutations have a worse prognosis and may need more intensive or different treatment.

Similar studies have been conducted in colorectal cancer. Chromosomal losses in colorectal tumour cells, particularly deletions of chromosomes 17p and 18q, have been found to be associated with worse prognosis.[17] An assay involving DNA amplification with the polymerase chain reaction and analysis of microsatellite markers to determine the status of chromosome 18q has been developed as a practical genetic test.[18] With this assay it has been shown that patients with stage II cancer and 18q allelic loss have a prognosis similar to that of patients with stage III disease.[18] This suggests that these patients should probably be grouped with the latter and given adjuvant therapy. By contrast, patients with stage II cancer and no allelic loss at 18q have been found to have a survival rate, both overall and relapse free, similar to that of patients with stage I disease.

In addition to influencing decisions about whether to give adjuvant therapy, molecular profiles may also influence choice of treatment. For example, specific

P53 mutations have been reported to be associated with de novo resistance to doxorubicin in breast cancer patients.[19] If confirmed, this observation would imply that in breast cancer patients with P53 mutations in the primary tumour this drug should be replaced.

Conclusions

The clinical benefit resulting from our greater understanding of the genetic pathogenesis of cancer could be important. It is unlikely that a single cancer gene could be used as a universal marker, but better characterisation of the genetic alterations underlying the more common tumours will hopefully provide a minimal set of molecular markers to be used for screening and early diagnosis of most cancers. Fulfilling this potential alone would result in a reduction in cancer morbidity and mortality, since more tumours would be curable at diagnosis. Other likely benefits in the near future include improved staging of cancer, greater prognostic accuracy, and better informed decisions about treatment. The ultimate goal, of curing most human cancers, will be more elusive and require a much better understanding of the biological consequences of the genetic alterations described in cancer cells.

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1 Nowell PC. The clonal evolution of tumor cell populations. *Science* 1976;194:23-8.
2 Kinzler KW, Vogelstein B. Lessons from hereditary colorectal cancer. *Cell* 1996;87:159-70.
3 Lengauer C, Kinzler KW, Vogelstein B. Genetic instability in colorectal cancers. *Nature* 1997;386:623-7.
4 Mao L, Lee DJ, Tockman MS, Erozan YS, Askin F, Sidransky D. Microsatellite alterations as clonal markers for the detection of human cancer. *Proc Natl Acad Sci USA* 1994;91:9871-5.
5 Sidransky D, Von Eschenbach A, Tsay YC, Jones P, Summerhayes I, Marshall F, et al. Identification of p53 gene mutations in bladder cancers and urine samples. *Science* 1991;252:706-9.
6 Hruban RH, van der Riet P, Erozan YS, Sidransky D. Molecular biology and the early detection of carcinoma of the bladder—the case of Hubert H Humphrey. *N Engl J Med* 1994;330:1276-8.
7 Mao L, Schoenberg MP, Scicchitano M, Erozan YS, Merlo A, Schwab D, et al. Molecular detection of primary bladder cancer by microsatellite analysis. *Science* 1996;271:659-62.
8 Sidransky D, Tokino T, Hamilton SR, Kinzler KW, Levin B, Frost P, et al. Identification of ras oncogene mutations in the stool of patients with curable colorectal tumors. *Science* 1992;256:102-5.
9 Caldas C, Hahn SA, Hruban RH, Redston MS, Yeo CJ, Kern SE. Detection of K-ras mutations in the stool of patients with pancreatic adenocarcinoma and pancreatic ductal hyperplasia. *Cancer Res* 1994; 54:3568-73.
10 Caldas C, Ponder BAJ. Cancer genes and molecular oncology in the clinic. *Lancet* 1997;349(SII):16-8.
11 Boyle P. Global cancer burden. *Lancet* 1997;349(SII):23-6.
12 Brennan JA, Mao L, Hruban RH, Boyle JO, Eby YJ, Koch WM, et al. Molecular assessment of histopathological staging in squamous carcinoma of the head and neck. *N Engl J Med* 1995;332:429-35.
13 Hayashi N, Ito I, Yanagisawa A, Kato Y, Nakamori S, Imoka S, et al. Genetic diagnosis of lymph-node metastasis in colorectal cancer. *Lancet* 1995;345:1255-9.
14 Pack S, Vortmeyer AO, Pak E, Liotta LA, Zhuang Z. Detection of gene deletion in single metastatic tumour cells in lymphnode tissue by fluorescent in-situ hybridisation. *Lancet* 1997;350:264-5.
15 Slamon DJ, Clark GM, Wong SG, Lewin WJ, Ullrich A, McGuire WL. Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. *Science* 1987;235:177-82.
16 Hartmann A, Blaszyk H, Kovach JS, Sommer SS. The molecular epidemiology of P53 gene mutations in human breast cancer. *Trends Genet* 1997;13:27-33.
17 Kern SE, Fearon ER, Tersmette KWF, Enterline JP, Leppert M, Nakamura Y, et al. Clinical and pathological associations with allelic loss in colorectal carcinoma. *JAMA* 1989;261:3099-103.
18 Jen J, Kim H, Piantadosi S, Liu Z-F, Levitt RC, Sistonen P, et al. Allelic loss of chromosome 18q and prognosis in colorectal cancer. *N Engl J Med* 1994;331:213-21.
19 Bergh J, Norbeg T, Sjogren S, Lindgren A, Holmberg L. Complete sequencing of the p53 gene provides prognostic information in breast cancer patients, particularly in relation to adjuvant systemic therapy and radiotherapy. *Nat Med* 1995;1:1029-34.

Lesson of the week

Two cases of retention of wooden foreign bodies in orbit of eye

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Beware of retained wooden foreign bodies associated with ostensibly minor injuries in the vicinity of the orbit

Missed intraorbital foreign bodies may lead to sight threatening and life threatening complications.[1] Although penetrating injuries of the orbit are usually obvious, cases of retention of intraorbital foreign bodies after apparently minor trauma have been reported and continue to be missed.[2 3] We present two cases of intraorbital wooden foreign bodies that were initially undetected. A high index of suspicion and a detailed history and examination are important in suspected cases of intraorbital foreign bodies.

Case reports

Case 1

A 20 month old girl presented to the paediatric accident and emergency department having sustained a fall indoors near a fireplace. A laceration of the right cheek was closed with sterile skin closures (3M, St Paul, MN) and she was discharged. Forty eight hours later she presented with persistent swelling and erythema of the cheek and lower lid. She was admitted and given intravenous antibiotics. Plain *x* ray films of the facial and orbital bones were unremarkable.

One week after the original injury an ophthalmic opinion was sought. Visual acuity tested with Teller acuity cards was appropriate for the child's age. Her right lower lid was swollen and the conjunctiva chemosed, and there was a purulent discharge arising from the region of the lateral canthus. There was no evidence of ocular perforation, and she had full extraocular movements. Topical chloramphenicol was prescribed and review in an outpatient clinic arranged. One week later the clinical signs had not resolved and abduction of the right eye was limited. The possibility of a retained foreign body was considered and she was admitted for examination under anaesthesia. Exploration showed a 2 cm wooden foreign body in the extreme aspect of the right lateral fornix, which on

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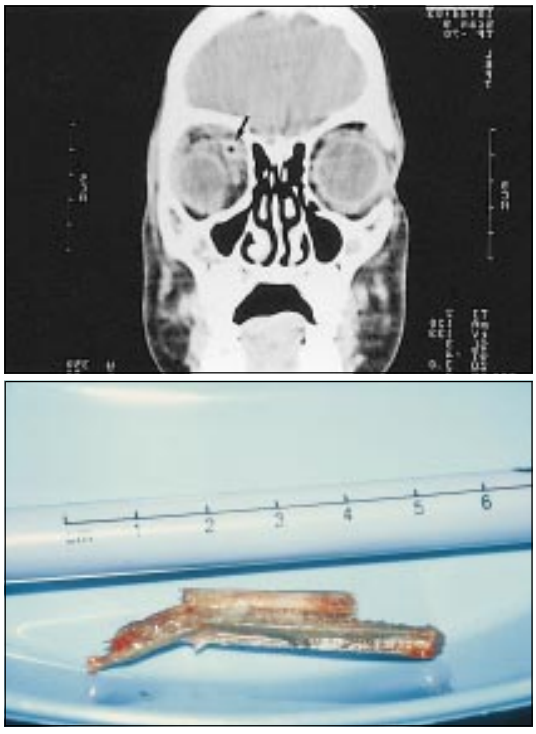
palpation extended along the lateral wall of the orbit. The foreign body was removed with toothed forceps. There was no evidence of scleral perforation. She was prescribed oral and topical antibiotics and discharged the next day. One week postoperatively the clinical signs and limitation of abduction had resolved.

Case 2

A 9 year old boy presented to the ophthalmic accident and emergency department after falling on to a bush while running in a wood. On examination a vertical laceration 1 cm long was noted on the bridge of his nose just right of the midline. His right upper lid was mildly swollen. Full ophthalmic examination including funduscopy gave normal results. Antibiotic ointment was applied to the skin wound, and he was discharged. Eight days later he presented with diplopia and marked swelling of the right upper lid. On assessment he was not feverish and had normal visual acuity on testing with Snellen charts. There was a purulent discharge from the original laceration site, a 3 mm right proptosis, and restricted elevation and adduction of the right eye. Computed tomograms of sections in the coronal plane and axial reconstruction showed a large opacity (9 x 6 mm), with a low attenuation centre extending deep into the superior medial aspect of the orbit (figure). There was no evidence of intracranial penetration. During exploration under general anaesthesia, a medial orbital abscess was drained and a piece of wood 5 cm long was removed from the abscess cavity.

Intravenous antibiotic treatment was started. Postoperative recovery was uneventful, and over 3 weeks the ocular movements recovered and the diplopia resolved.



(Top) Computed tomogram of case 2 showing location of wooden foreign body (arrow) in superior medial aspect of right orbit. (Bottom) Foreign body removed

Comment

If wood has a sharp end and is elongated it can penetrate deep into the orbit and the intracranial cavity through a small entry wound.[1 4] Unlike metal, wood is prone to snap and therefore the foreign body may be concealed within the orbit. The resistance of the sclera and the displacement of the globe often protect the eye from perforation.[5] This can, as in our two cases, result in a foreign body that is well buried and an eye that seems normal. Despite being serious some penetrating injuries in the vicinity of the orbit can initially seem trivial. Early clinical signs suggesting a foreign body in the orbit may be displacement of the globe, persisting inflammation, and chemosis. Limitation of ocular movement with diplopia should also arouse suspicion but may not become evident until later.[2] Decay of retained organic material and supervening infection almost invariably causes an abscess. This may affect the function of the optic nerve with loss of vision.[6] Furthermore, the infection may spread intracranially, either directly or through the ophthalmic vein, and lead to an intracranial abscess and cavernous sinus thrombosis. Despite their removal some wooden intraorbital foreign bodies may cause recurrent orbital inflammation because of retained debris. This may occur long after the original trauma has been forgotten.

Wood has a density similar to air and fat and can be difficult to distinguish from soft tissue in both a plain *x* ray film and a computed tomogram.[1–3 7] In case 2 the abscess helped delineate the foreign body in a computed tomogram but did not show its full size. Magnetic resonance imaging is a better method of investigation in cases of a suspected organic intraorbital foreign body.[3 7]

In summary, clinicians should be alerted to the possibility of retention of an intraorbital foreign body in all patients presenting with periorbital trauma. The clinician should also obtain a careful history of the type of injury and should examine the patient in detail. In cases where a wooden foreign body is suspected investigation by magnetic resonance imaging is the preferred procedure.

1 Mutlukan E, Fleck BW, Cullen JC, Whittle IR. Case of penetrating orbitocranial injury caused by wood. *Br J Ophthalmol* 1991;75:374-6.
2 Macrae JA. Diagnosis and management of a wooden orbital foreign body: case report. *Br J Ophthalmol* 1979;63:848-51.
3 Green BF, Kraft SP, Carter KD, Buncic JR, Nerad JA, Armstrong D. Intraorbital wood. Detection by magnetic resonance imaging. *Ophthalmology* 1990;97:608-11.
4 Greany MJ. Bamboo orbital foreign body mimicking air on computed tomography. *Eye* 1994;8:713-4.
5 Hansen JE, Gudeman SK, Holgate RC, Saunders SA. Penetrating intracranial wounds: clinical implications of computerised tomography. *J Neurosurg* 1988;68:752-6.
6 Weisman RA, Savino PJ, Schut L, Schatz NJ. Computed tomography in penetrating wounds of the orbit with retained foreign bodies. *Arch Otolaryngol* 1983;109:265-8.
7 Specht CS, Varga JH, Jalali MM, Edelstein JP. Orbitocranial wooden foreign body diagnosed by magnetic resonance imaging. Dry wood can be isodense with air and orbital fat by computed tomography. *Survey Ophthalmol* 1992;36:341-4.

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Correction

Hypopituitarism after coronary artery bypass grafting

An error occurred in this article by Davies and Scanlon (28 February, pp 682-5). The third sentence describing case 2 should have read: "Venous grafts were inserted to the first diagonal branch of the left anterior descending artery, the first and second obtuse marginal arteries, and the posterior descending artery [not the patent ductus arteriosus]."