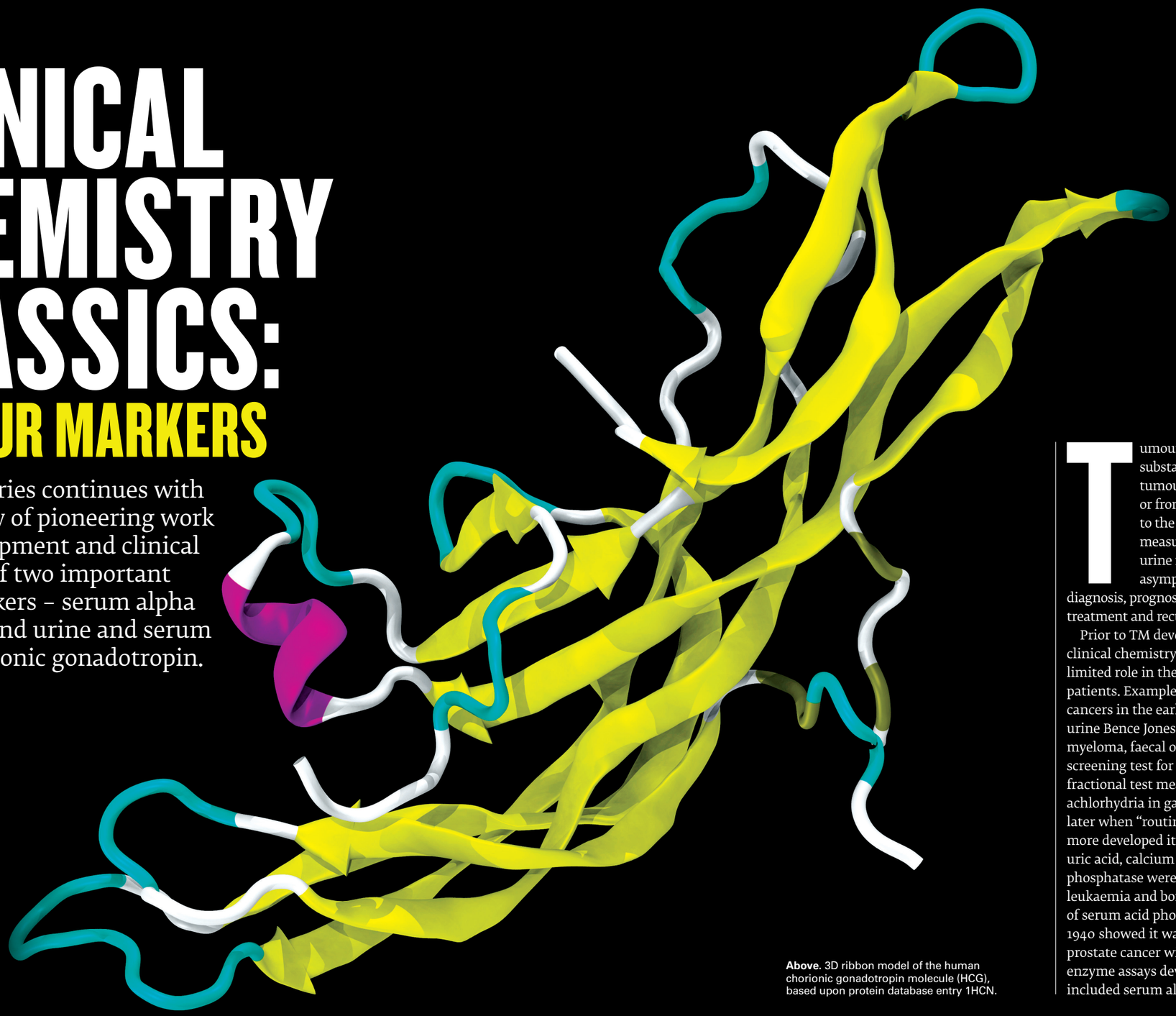
CLINICAL CHEMISTRY CLASSICS: TUMOUR MARKERS

This short series continues with a brief review of pioneering work in the development and clinical application of two important tumour markers – serum alpha fetoprotein and urine and serum human chorionic gonadotropin.



Above. 3D ribbon model of the human chorionic gonadotropin molecule (HCG), based upon protein database entry 1HCN.

Tumour markers (TMs) are substances released by a tumour into blood or urine, or from the host in response to the tumour. Their measurement in serum or urine may be used in screening asymptomatic patients, for diagnosis, prognosis, monitoring treatment and recurrence detection.

Prior to TM development in the 1960s, clinical chemistry laboratories played a limited role in the investigation of cancer patients. Examples of chemical tests in cancers in the early 20th century included urine Bence Jones protein in multiple myeloma, faecal occult blood as a screening test for colorectal cancer and fractional test meals to identify achlorhydria in gastric cancer. Decades later when "routine" manual tests were more developed it was found that serum uric acid, calcium and alkaline phosphatase were of clinical value in leukaemia and bone metastases. Analysis of serum acid phosphatase reported in 1940 showed it was significantly raised in prostate cancer with metastases. Other enzyme assays developed in the 1950s included serum alanine and aspartate transaminases, which were raised in hepatocellular carcinoma.

Tumour markers

Pioneer studies by Ludwik Gross in 1943 and EJ Foley a decade later established the existence of tumour specific transplantation antigens in chemically- or virally-induced animal tumours. Research then proceeded to human tumours and with advances in immunology, combined with intensive studies using novel antibodies and methods, such as double diffusion, gel filtration and immunoelectrophoresis (IEP), a number of tumour associated antigens were identified for more specific tumours during the following 20 years, notably for liver (1964), colon (1965), ovaries (1969) and breast (1971). It was proposed that specific tumour antigens could act as markers for the diagnosis, prognosis and management of certain cancers. In order to formulate TM assays in body fluids, specific tumour tissue extracts were used as immunogens to produce reactive antibodies, with the methods described for qualitative studies. Early quantitative serum methods used immunodiffusion, radioimmunoassay or

ELISA but with advances in antibody linkage technology, more sensitive assays became available and with increased specificity using monoclonal antibodies.

Ideal criteria for tumour markers

The ideal tumour marker assay should have high sensitivity and a low rate of false negatives, high specificity and low rate of false positives, show a positive correlation with tumour volume and extent and the clinical value has been validated by approved prospective trials. The assay should be relatively non-invasive, inexpensive, simple and automated. More than 20 substances have been identified as tumour markers and are in clinical use. However, unfortunately none to date fulfils all these criteria as an ideal tumour marker, and it is surprising that few new TMs have been introduced to clinical practice during the last 30 years. However, the two tumour markers reviewed here have been shown to be clinically useful in diagnosis, treatment and prognosis.

Alpha fetoprotein (AFP)

AFP is a 70kDa glycoprotein, which is homologous to albumin and may perform some of the functions of albumin in the foetal circulation. In 1956, Bergstrand and Czar in Stockholm, identified a new alpha1 globulin in foetal serum using paper electrophoresis. In 1963, Abelev and colleagues in Moscow reported that in chemically-induced and transplantable hepatomas, mice and rats synthesised and secreted AFP into blood. In the following year, Tatarinov reported raised serum AFP in six patients with hepatocellular cancer (HCC). In 1967, Abelev and associates in a landmark article described more fully the clinical importance of AFP in HCC in a larger study using agar precipitation and immunoelectrophoresis (IEP), and reported that serum AFP was also raised in non-seminomatous germ cell (NSGC) tumours of testis or ovary but not in some other testicular tumours. These results have been confirmed in many other studies and serum AFP is now regarded as a first line biochemical test in the diagnosis of HCC, combined with abdomen ultrasound and with serum HCG for NSGC tumours. With a reference range of 0-12ng/ml, a serum AFP greater than 500 ng/ml is regarded as diagnostic of HCC and a failure to clear AFP following surgical liver resection and chemotherapy indicates a poor prognosis. Raised levels may also be found in hepatitis, cirrhosis and biliary tract obstruction and hepatitis B & C are risk factors for the development of HCC.

AFP is an important tumour marker as HCC is the fifth most common cancer and the third significant cause of cancer mortality in the world, with a poor five-year survival rate of only 7%.

Analytical methods used have ranged from IEP, radioimmunoassay, enzyme immunoassay and immunoradiometric assays during the late 20th century, to the more sensitive types of assay, such a chemiluminescent microparticle inmmunoassay.

The measurement of serum AFP is also highly relevant in screening pregnancies for the detection of open neural tube defects, notably spina bifida with raised results and for Down's syndrome with lower results. The former dates from 1972 with a study that found greatly increased AFP concentration in amniotic fluid and the same Edinburgh group reported an increase in maternal serum AFP the following year. Screening is combined with confirmatory ultrasonography.

Several studies reported in 1984 identified an association between low maternal serum AFP in Downs's syndrome and a group led by Howard Cuckle at St Bart's, London proposed its potential value in screening. This was confirmed in many further studies and maternal serum AFP and HCG are now used worldwide for Down's screening after the first trimester as two of the biochemical markers in the "quadruple" test.

Human chorionic gonadotropin

The isolation of adrenalin and secretin in the early years of the last century and the concept of hormones as chemical messengers, as proposed by the British physiologist Ernest Starling in 1905, encouraged a surge of active endocrine research by many groups, notably in Germany during the next three decades. This led to an improved understanding of the human ovarian cycle and the hormonal changes that take place in pregnancy. In 1903, Ludwig Fraenkel, professor of gynaecology at the Women's Hospital, Breslau provided experimental proof of the endocrine function of the corpus luteum. With the ready availability of placental tissue, studies by Bernhard Aschner (1912) and Otto Fellner (1913) and Japanese scientist Toyoichi Hirose (1920) showed placental tissue extracts had stimulatory effects on the genital tract, ovulation, and corpus luteum and progesterone production in guinea pigs and rabbits respectively. This clearly demonstrated that the placenta had an endocrine function supporting pregnancy.

Urine HCG as a pregnancy test

In 1928, German endocrinologist Selmar Aschheim and German-born Israeli gynaecologist Bernhard Zondek working at the Berlin Charite, showed that in pregnancy, women produced a high concentration of a gonad-stimulating substance (HCG) in their urine which activated receptors on the gonads of mice. Although the A-Z test took five days, was expensive and required special animal house facilities it had a reported error rate of <2% and was sensitive detecting HCG seven to 10 days after a missed period. A reference station was established in Edinburgh with a postal sample service in 1930 and the procedure was soon used in some London hospitals. In 1931 Maurice Friedman and Maxwell Lapham at the University of Pennsylvania developed a similar technique with large female adult rabbits and the condition of the ovaries was examined after just one day and by 1935 this became the method of choice in London and the United States.

HCG as a tumour marker

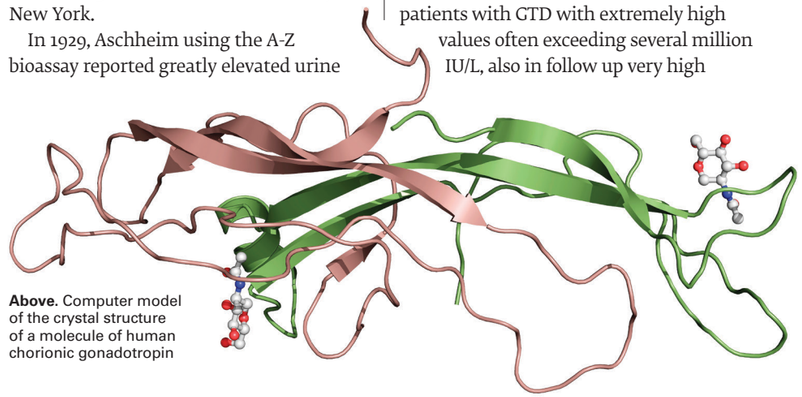
HCG is a 36.7kDa glycoprotein produced by the trophoblast tissue of the placenta in pregnancy and a number of other sites in malignant conditions. HCG stimulates the ovarian corpus luteum to secrete progesterone until the placenta takes over production to sustain pregnancy, it also promotes cell fusion of cytotrophoblast to syncytiotrophoblast and maternal myometrial spiral artery angiogenesis.

HCG is a heterodimer with two glycosylated sub units, the alpha sub unit structure is common to other gonadotropins, for example luteinising hormone, but the beta sub unit of 145 amino acids is unique to HCG. The amino acid sequence of both sub units was established in 1975 by Francis Morgan and colleagues at Columbia University, New York.

In 1929, Aschheim using the A-Z bioassay reported greatly elevated urine HCG results in two forms of gestational trophoblastic disease (GTD), choriocarcinoma and hydatidiform mole and in these cases urines often required 1/200 dilution. Choriocarcinoma was first described in Germany by Hans Chiari in 1877 with histological studies reported by M Saengers (1889) and six years later by Felix Marchand. It appears hydatidiform mole was recognised in ancient medicine but described in more detail by Madame Boivin in 1827, who recognised that the hydatids are grape like cystic dilatations of the chorionic villi. Tumours of trophoblastic origin may arise in the uterus, placenta or gonads and tend to be highly malignant and may spread to the lungs and brain. Gestational choriocarcinoma (GC) is rare and occurs in 1/20-50,000 pregnancies, according to conflicting studies, but prior to the 1950s treatment was limited to surgery and radiation and the prognosis was poor. GC has a special place in cancer history, as it was the first condition successfully treated by chemotherapy when in 1958 Roy Hertz and Min Chiu Li at the National Institute of Health, Maryland developed the use of the folic acid inhibitor, methotrexate with great success.

Clinical applications of HCG as tumour marker

The most often adult quoted reference range for serum HCG is <5 IU/L. Serum HCG is used in the follow up and monitoring patients with GTD with extremely high values often exceeding several million IU/L, also in follow up very high



Above. Computer model of the crystal structure of a molecule of human chorionic gonadotropin

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results may indicate treatment failures. HCG concentration tends to correlate with tumour volume allowing accurate titration of chemotherapy. The most adult quoted reference range is <5 IU/L.

Serum HCG in combination with AFP and Lactate dehydrogenase is recognised as the best validated prognostic markers for diagnosis, follow up and monitoring germ cell tumours (seminomas, NSGCT and extragonadal tumours).

It is important to distinguish seminomas from teratomas, as each is treated differently with chemotherapy or surgery respectively. In seminomas HCG may range from 10-2000 IU/L. In NSGCT HCG may range from 5-1000 IU/L and depends on stage of the tumour. CSF HCG is more increased in primary intracranial germ cell tumours compared with serum HCG.

Analysis of HCG

HCG exists in multiple forms in serum as intact HCG, Free Beta subunit, Beta core fragment, nicked HCG and hyperglycosylated HCG. Consequently, when used as a tumour marker the HCG assay should detect all main forms, and laboratories should be aware of the characteristics of the assay in use in clinical interpretative medicine. The Beta core fragment is the main form in urine but hyperglycosylated HCG is main urine form in early pregnancy.

The AZ urine HCG test was used extensively with a number of modifications, most notably in 1941 by Eleanor Delfs, the distinguished professor of Obstetrics at Johns Hopkins University and later pioneer HCG researcher at Wisconsin Medical College. Delfs developed a serum HCG bioassay injecting serum extracts into female rats and measuring uterine weight.

Bioassays continued until the first immunoassays were developed in 1960, this followed the typical development pattern of peptide immunoassays with first complement fixation and in the same year an haemagglutination inhibition assay for urine HCG devised

The pioneering work laid the foundations for two of the most useful tumour markers to date

by Lief Wide and Carl Gemzell in Stockholm, the reactants were incubated in tubes for two hours and read visually. In 1962, a group led by Jennifer Robbins developed a similar latex agglutination method version using coated latex beads for urine HCG. The Gravindex pregnancy test was based on this latex agglutination inhibition principle.

In 1965, a radioimmunoassay was developed by a team at Charing Cross Hospital Medical School, led by CE Wilde. Charing Cross became a major oncology centre for GTD under the leadership of Prof Kenneth Bagshawe during this period and in 1969 he reported an improved HCG radioimmunoassay. Charing Cross was also notable for pioneering chemotherapy agent developments, including multidrug combinations under the leadership of Edward Newlands, in GTD, testicular, ovarian cancer and brain tumours.

Early commercial kit methods for urine HCG using the agglutination methods were compared and critically reviewed by Derek Watson in 1966 and found to be suitable for detection and monitoring GTD. In 1972, a radioimmunoassay specific for beta HCG was developed and was shown to be clinically useful in monitoring chemotherapy in GTD and follow up of terminated molar pregnancies. The introduction of monoclonal antibodies into immunoassays from 1975 led to a range of two antibody immunometric assays using high sensitivity fluorimetric and chemiluminescent tracer detection assays developed over the last few decades. Reported potential sources of error include lack of specificity-some trophoblastic tumours secrete nicked HCG which is not measured in all assays, due to the very high HCG concentrations often encountered the "high dose hook" effect produce falsely low results and samples require dilution. In addition the presence of heterophilic antibodies may cause errors. Point-of-care and commercial devices for urine pregnancy testing generally are based on a sandwich ELISA dye detection principle and are qualitative only, it is essential they detect hyperglycosylated HCG.

Concluding comments

AFP and HCG have been shown to be remarkable glycoproteins with combined importance in Down's syndrome screening in maternal serum and in monitoring NSGCT. Serum AFP is the first line tumour marker in HCC and serum HCG measurements are essential in the management of GTD.

The pioneering work of the scientists, Abelev and Tatarinov, and Aschheim and Zondek, laid the foundations for their use as two of the most useful tumour markers to date. BMS

Stephen Clarke is a retired IBMS Fellow. He previously worked in clinical chemistry at Southmead Hospital, Bristol.
To see the references, view the article online at **thebiomedicalscientist.net**