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British Journal of Biomedical Science in 2017: What have we learned?

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ABSTRACT

In 2017 the British Journal of Biomedical Science published 35 articles in the various disciplines that comprise biomedical science. These were 6 reviews, 22 original articles, 6 'In Brief' short reports and one guideline. Of these, the majority were in clinical chemistry (one review, six data papers), microbiology (one review, four data papers), cellular pathology (four data papers) and virology (one review, two data papers). There were two data papers in transfusion science, whilst haematology, cytopathology and immunology were each represented by one review and one data paper. Reflecting the increasing complexity of the laboratory, five data papers crossed barriers between traditional disciplines, and so may be described as multidisciplinary. The present report will summarise key aspects of these publications.

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Introduction

The *British Journal of Biomedical Science* is the leading international journal focusing on practice, research and education in all aspects of biomedical science as it applies to the diagnosis and clinical management of human disease. This generally focuses on the practice of routine biomedical/clinical science in NHS hospitals, but can also embrace developing methods, cells and molecules, such as in tissue culture, pharmacology and molecular genetics. The growing importance of the latter is demonstrated by the fact that of 28 data papers published this year, just over half (53.6%) used techniques in RNA and/or DNA. This is considerably higher than the rate in the 34 data papers (38.2%) published in 2016. In issue 1 of 2016, the Journal published an article summarising work published during 2015 [1]. Similarly, issue 1 of 2017 included an article summarising work published during 2016 [2]. The present communication aims to continue this process with a summary of those aspects of papers published during 2017 which, in the opinion of the Editor, report the most practical advances in biomedical science, classified by major discipline.

Cellular pathology

Vav3, with roles in signal transduction and metastatic invasion, is over-expressed in a variety of cancers, and encodes a soluble protein product (Vav3) [3], measurable by ELISA. Tan et al. [4] showed increased serum and tissue Vav3 in stomach cancer, with serum levels some 40% higher in lymphatic metastases, and that serum levels fall to normal after surgical excision. Thus Vav3 could be a new cancer marker that may enter routine practice. Mohs micrographic surgery (MMS) is a technique that aims to remove a skin tumour and deep circumferential tissue to ensure all neoplasia has been excised. Using 279 tissue samples from 10 facial sites, Orchard et al. report two new innovative tools that bring benefits of automation, speed and efficiency to the preparation of frozen sections from MMS with high levels of accuracy (93.5%) and precision/reproducibility (96.5%) [5]. Non-small cell lung cancer is a leading cause of cancer deaths worldwide, non-tobacco links being genes such as for epidermal growth factor receptor and anaplastic lymphoma kinase (*ALK*). Mohamed et al. used immunocytochemistry on tumours from 92 patients, of whom 12 were positive for ALK protein expression [6]. These 12 were more likely to be women, to be never-smokers, and to have a metastatic lesion. They conclude that this method is a reliable alternative to FISH. Ovarian cancer has a number of forms, some arising from epithelial, other from germ or stromal cells. Two genes, *WT1* and *PAX8*, may be useful in differentiating these alternative forms. Rhodes et al. report that the majority (86–87% respectively) of tumours of a serous origin were positive for immunohistological expression of the genes: endometriod tumours were less likely to be positive [7].

Clinical chemistry

We should in theory excrete all our nitrogenous waste in urine and faeces. However, in certain pathological conditions, some (as ammonia) may be excreted via the lungs.

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This commentary is the object of a journal-based-learning event for continuing professional development.

Our Portuguese colleagues reported their development of a method for determining ammonia in expired breath, and used it in a study of patients on dialysis [8]. Perhaps unsurprisingly, breath ammonia correlated with blood urea ($r^2 = 0.55$, $p < 0.001$), leading to the suggestion that this method may, in certain circumstances, be an alternative to standard serum biochemistry. Although few would argue against a powerful role for overweight and obesity in the development of type 2 diabetes, this alone does not account for all disease. Rizvi et al. tested the hypothesis that two genes, *Multidrug resistance 1* (whose function is clear) and *CYP46A1* (linked to cytochrome P450) have roles in diabetes [9]. Although the latter was linked to the disease, the former was not, leading to the speculation that measurement of polymorphisms in *CYP46A1* may contribute to determining the risk of developing type 2 diabetes.

A common treatment for nephrotic syndrome is steroids. However, responses vary from patient to patient for reasons that have, until recently, been unclear. One potential explanation is the effect of polymorphism in genes for certain metabolic enzymes. This paper from Egypt [10] found that one such polymorphism (in *ABCB1*) was modestly effective in determining responses to this drug, leading to the possibility that it may help direct treatment. If so, analysis of *ABCB1* may enter routine practice. Those who cannot obtain their nutrients via the digestive system must be fed intra-venously by the process of parenteral nutrition. The report by Fung et al. [11] underlined the importance of measuring serum phosphates, as low levels are surprisingly common, and so should be checked regularly, perhaps daily, so that deficiencies can be detected and rectified. Of 57 patients, serum phosphate was 14.5% higher in those 26 who subsequently died. Brown reviewed a number of significant developments in toxicology within clinical laboratories, both with the available instrumentation and in the range of compounds abused by the drug-using communities [12]. He summarised developments in the regulation of forensic science in the UK which may in time impact on clinical toxicology. In the search for better laboratory methods, Fan et al. reported their development of an immunoassay that can measure two molecules at the same time [13]. An interesting part of this is the use of two fluorescent rare-earth elements (europium and samarium) to label monoclonal antibodies, and magnetic nanoparticles as a separation step. This is possible as the two elements have different emision wavelengths (615 and 642 nm, respectively). This method could be adapted for the immunoassay of almost any two analytes. Hypercalcaemia and hypercalcuria are risk factors for the development of nephrolithiasis (kidney stones). However, by definition, renal disease is a major confounder of an accurate diagnosis, and so a 24-h sample may be needed. Our colleagues from China showed that a fasting urinary calcum to creatinine ratio correlates very strongly with 24 h calcium excretion ($r = 0.77$) and so could be a simple, quick and convenient test for the diagnosis of kidney stones [14]. The miR-302 family of molecules are post-transcription regulators of cell cycle progression. As one member, miR-302b, targets the regulation of the epidermal growth factor receptor, it has attracted the interest of oncologists. It is therefore interesting to note that Fu et al. investigated levels in those suffering a myocardial infarction [15]. Not only were levels of this molecule 3.8 times higher in those with the condition, levels also correlated very strongly with established biomarkers CK, CK-MB and troponin I (r = 0.79, 0.79 and 0.92 respectively). The authors speculate that miR-302b will out-perform standard biomarkers. If so, this may be among the first such molecules to enter the routine service.

Cytopathology

Human papilloma virus (HPV) has several isotypes, the most dangerous of which confer a risk of cervical cancer, and as such is a major public health issue. There is clear evidence of a reduction in the incidence of cancer where HPV testing is used. As cervical cytology is becoming increasingly rare, Crossley & Crossley reviewed its effectiveness and role in cervical screening [16]. Diagnosis of a malignant or benign pleural effusion based on cytology has poor senstivity and specificity, possibly due to the sampled cells being unrepresentative of the complete pathology. The advance reported by Eltorgoman et al. is that combining cytology with the ratio between two fragments of DNA (Alu 115 and Alu 247 repeats) brings the specificity and diagnostic accuracy to 100% [17]. Altered levels and expression of microRNAs are present in a wide range of diseases, including cancer, and may have regulatory roles. Our colleagues from China [18] showed that expression of miR-199a-3p in papillary thyroid cancer tissue (mean 7.1 units) is markedly lower than in normal thyroid tissue (mean 31.4 units), and accordingly may be a new marker for this disease. Furthermore, low levels were linked to lymph node ($p = 0.036$) and distant metastases ($p = 0.002$).

Haematology

Although a spectactularly effective therapeutic, warfarin (in common with many drugs) has activity outside its primary function. Donaldson & Harrington review these effects, mostly on bone mineralisation and calcium homeostasis (which may lead to osteoporosis and heart valve pathology) and damage to the embryo [19]. In 2016, Gurney reviewed the importance of platelets [20]. Moore and colleagues continued this theme, showing that they have many other functions, such as in wound repair, potentially due to intra-platelet growth factors [21]. This paper reported that platelets retain clinical use in this setting after storage for 5–8 days

Immunology

Karri and Sheela reviewed interactions between pro-inflammatory and anti-inflammatory cytokines, and how these influence and so regulate an immune response [22]. An example of this is the interaction between IL17 and IL23 and certain T lymphocytes with regulatory and helper functions. Far from being academic, this informs our knowledge of the pathogenesis of autoimmune disease and so may provide new diagnostic and/or management opportunities. Abdul-Maksoud et al. reported [23] low serum levels of microRNA MiR-210 in rheumatoid arthritis (RA) that correlated inversely with TNF-α and IL-1β, and increased MiR-155 that correlated positively with TNF-α and IL-1β. They concluded that these MiRs are independent diagnostic markers for RA, out-performing several routine indices (ESR, CRP, rheumatoid factor), and reflect disease activity. Thus miR-210 and miR-155 might serve as non-invasive biomarkers for the diagnosis of RA.

Microbiology

Campylobacter, of which there are 29 species, is linked to a number of disease, notably those of the intestines. Last year, Nakajima and colleagues showed the presence of genes coding for catalase and catalase-like proteins that, according to the authors, may provide the organism with a method for protecting itself from toxic oxygen stress [24]. Casey and colleagues continued research into the biology of this genus, with a review focussing on sources, reservoirs, and laboratory growth and detection (such as by molecular genetics) [25]. Similarly, we recently published articles on *Helicobacter pylori*, an organism linked to a variety of diseases of the stomach, the most serious being cancer [26,27]. This year we carry work showing that a major deteriminant of its pathogenicity is a gene, *cag*, and its product. Our colleagues showed that alternative isotypes of *cag* are associated with different forms of gastic disease [28]. The strongest link is with *cagE*, which brings odd's ratio's of 5.0 and 3.0 for peptic ulcer disease and for gastric cancer, respectively. Integrons are gene acquisition systems commonly found in bacterial genomes that play a major role in the dissemination of antibiotic resistance. Our colleagues from Egypt reported that *Pseudomonas aeruginosa* resistance to biocides is linked to the presence of integron 1, and so may be important in multidrug resistance [29]. The genetics theme continued with a report on *mecA*, and its product, a key determinant of methicillin resistance. Cao et al. compared standard phenotypic detection of *mecA* in MRSA with fluorescent PCT, finding 98% concordance in sputum samples, and 100% in blood, urine and other body fluids, paving the way for an expansion of the use of method [30]. Antibiotic resistance is a growing problem, and accordingly the need to determine its extent is vital. Ewing et al. presented a score for determining the relative resistance of non-mucoid *Pseudomonas aeruginosa* to common antibiotics [31]. Validating their score clinically (RRI correlates with lung function [$r = -0.44$] and number of days on intra-venous antibiotics [$r = 0.4$]), they suggested it may be of benefit in quantifying antibiotic resistance. This provides a perspective on the report last year which demonstrated a laboratory method for assessing the virulence of this organism [32].

Transfusion science

Nuclei acid testing is a crucial part of screening donor blood for human immunodeficiency virus, and hepatitis B and C viruses, a luxury not always available to those on restricted budgets. Our colleagues from India reported their experience of using PCR kits to develop a semi-automated viral detection system that improves cost-effectiveness in both high and low volume blood banks [33]. *RHD* and *RHCE* code for RH blood group antigens. Mutations in *RHD* lead to a variant, DEL, resulting in 22-36 D molecules per red cell, compared to 30,000 copies on the surface of normal D red cells and 1500–7000 sites on cells with the weak D variant. Although our colleagues from Thailand found low levels of miR-98 in DEL and D-negative samples compared to D-positive cells, this difference was not statistically significant, suggesting other mechanisms must be responsible for the expression of the D molecule [34].

Virology

Members of the virology advisory panel refreshed and extended our knowledge of the most pathogenetically important viruses, these being hepatitis B and C, measles, HIV, respiratory syncytical virus, norovirus, papillomavirus and genital herpes simplex virus. Their article also described the value of the laboratory in diagnosis, genetic techniques, point of care testing, and recent developments (e.g. Ebola) [35]. Last year the Journal published a methods comparison paper of kits for the rapid diagnosis of influenza [36]. This year our colleagues from Ireland reported that the Xpert assay for noravirus has excellent performance criteria compared with a reference RT-PCT method, and out-performs an alternative assay method [37]. This is important as this virus accounts for >90% of cases of gastroenteritis and is a major issue in hospitals and care homes. Although multiplex PCR is a powerful technique, it is imperfect. Barratt et al. reported a new method that allows greater multiplexing as multiple targets can be assessed simultaneously, and so provides a clinical validation allowing a throughput of respiratory samples and the automated analysis of results [38].

Multidisciplinary

In this section, we look at papers whose material is relevant to more than one of the major disciplines, such as the following three, which consider different aspects of

liver disease. Hepatitis C infection is a major risk factor for hepatocellular cancer (HCC), which is linked to *c-Myc* and *p53*. Of 120 patients with this infection, of whom half had HCC, Attalah et al. found that the sensitivity of serum c-Myc and p53 proteins for detecting HCC were higher than that for alpha feto-protein. When both gene products were combined, sensitivity was 100%, suggesting these may be a new diagnostic tool [39]. Routine liver function tests and a platelet count, although abnormal, were unable to discriminate the groups. Liver fibrosis is a major concern in those infected with hepatitis viruses B and C, alone or in combination. Our collegues from Egypt also showed, using Western blotting and ELISA, that although levels of soluble collagen type III and MMP-1 levels were no different in mono- or co-infection, levels of both were higher in those with more severe fibrosis [40]. Cirrhotic liver failure in hepatitis C virus infection is a dominant chronic pathology, but acute bleeding from oesopheal varices can be acutely life-threatening. Farid et al. presented a new score for predicting risk of this haemorhage (a combination of haematology and biochemistry indices: platelet count, prothrombin time and alpha-fetoprotein, but not routine liver function tests or haemoglobin) that out-performs eight other scoring systems [41].

Perhaps the most urgent call for a laboratory scientist is in acute heart disease: last year the potential value of transforming growth factor beta as a marker of acute myocardial infarction was compared to standard markers such as troponin T [42]. Despite best medical care, up to 30% of heart transplants recipients experience a rejection episode in the first year. The gold standard of myocardial biopsy is far from ideal, leading to the search for alternatives, such as troponins. Guo et al. presented data showing that miR-29, alongside total white blood cell count, lymphocyte count, cTnI and NT-proBNP, should be considered a new marker of rejection [43]. Although the use of contrast media in diagnostic procedure is a valuable technique, it may cause renal damage. Our colleagues from China presented data on a new immunoassay that can determine urine levels of renal markers cystatin C and β_2-microglobulin simultaneously [44]. This is possible as antibodies are labelled with europium and samarium, which fluoresce at different wavelengths. This method compares well with separate commercial assays for each marker, so may enter routine laboratory practice. Notably, Fan et al. also reported the use of these two lanthanide cations for the simultaneous detection of pepsinogens [13].

Table 1. miRNA papers recently published in the Journal.

Refs.	miRNA species	Advance reported
[48]	miR-330–5P	The miR can silence the gene expression of TIM-3 (a marker highly expressed on leukaemic stem cells) *in vitro*
[49]	miR-124	Compared to pancreatitis and healthy controls, low serum miR is present in pancreatic cancer and predicts poor overall survival
[50]	miR-21	Increased expression of the miR is present in osteosarcoma tissue and predicts poor overall survival
[51]	miR-146a	No link between tissue polymorphism in the miR and gastric carcinoma
[15]	miR-302b	Plasma miR-302b correlates positively with cardiac markers, and may be a better indicator of myocardial infarction
[18]	miR-199a-3p	Reduced expression of the miR in tissue from thyroid cancer tissue compared to normal thyroid tissue
[23]	miR-210 and miR-155	Low serum miR-210 but raised serum miR-155 in rheumatoid arthritis that correlate inversely/positively with TNF-α and IL-1β. Links with disease activity
[34]	miR-98	No link between the expression of the miR and expression of D antigen on red cells
[43]	miR-29	Increased blood levels of the miR are linked with the likelihood of heart transplant rejection, correlate with troponin I, and fall with time after the transplant

Guidelines

The Journal is glad to serve laboratory scientists by publishing practice guidelines from relevant professional bodies. Although not an IBMS body, the Association of Biomedical Andrologists is the leading UK professional group for scientists, and has strong links to the IBMS. Their guideline offered advice and recommendations regarding the assement of semen and the viability of sperm [45].

Are miRNAs the new Bob Dylan?

The importance of molecular genetics in laboratory science is such that of 28 papers with original articles published this year, just over half (15) used methods in DNA and/or RNA. microRNAs (miRNAs; short [18–25 nucleotides, hence micro] non-coding nucleotide sequences) are one of the more interesting and possibly important entrants into laboratory science in the last few years [46, 47]. Last year the Journal published four articles on these molecules [48–51], and we continued the trend with five such articles this year [15,18,23,34,43]. These nine papers, and their major findings, are summarised in Table 1. With a growing number of clinical uses for these molecules, it is perhaps only a matter of time before they enter the routine service. It is also possible that miRNAs may also be a new form of therapy [52], possibly deliverable to target cells by monoclonal antibodies or viruses [53], further justifying their entry into routine pathology laboratory work.

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