

CHEST & HEART

NEWS LETTER

Morbidity and mortality in cystic fibrosis (CF) are dominated by the complications and consequences of chronic infection and inflammation. Acute and chronic inflammation secondary to bacterial infection results in injury to respiratory epithelium and subsequently to alveolar tissue. Despite aggressive antibiotic therapy for acute exacerbations and disease-modifying agents to control the impact of chronic infection, inflammation continues relatively unchecked. This results not only in a local inflammatory process but also in secondary systemic complications. Chronic systemic inflammation has a significant impact on other organ systems and may contribute to the development of related conditions, such as undernutrition, CF-related bone disease, and CF-related diabetes mellitus. Systemic vasculitis and CF-related joint disease are also likely to be related to the systemic inflammatory effect driven by chronic pulmonary inflammation and infection. CF is a disease of epithelial cells and therefore involves other organ systems apart from the lung where epithelial cells are present. Pancreatic inflammation is a major factor in the development of fat malabsorption and diabetes mellitus, whereas biliary tract disease results in several disorders affecting the liver, particularly cirrhosis and portal hypertension.

In many of these situations, CF transmembrane regulator (CFTR) dysfunction in the involved epithelium may interact with the consequences of systemic inflammation to contribute to the disease process.

Nutrition and Intermediary Metabolism

The causes of undernutrition in people with CF are multifactorial (Table 1). It is likely that pancreatic injury begins in utero with mucous plugging of pancreatic ducts. This causes a secondary inflammatory process with loss of exocrine pancreatic tissue,

bicarbonate deficiency, and subsequently islets of Langerhans. The pancreatic disease in CF contributes to maldigestion and malabsorption by reducing the presence of pancreatic enzymes because of destruction of exocrine pancreas. The pancreas is quite sensitive to the degree of CFTR dysfunction. Pancreatic enzyme deficiency associated with pancreatic insufficiency is a key factor but in the majority of cases can be corrected with the use of pancreatic enzyme replacement therapy (PERT). Undernutrition is seen in around 20% of children and adults with CF (Fig. 1). The main reasons for not achieving good nutritional status is poor adherence to PERT and energy supplementation and poorly controlled lung disease. Appropriate intervention should be designed for each individual to optimize both PERT and energy intake.

Poorly controlled lung disease can contribute to undernutrition in several important ways. Impaired lung function is associated with an increase in oxygen costs of breathing and this may increase resting energy expenditure in people with CF. Chronic inflammation is associated with increased resting energy expenditure. The combination of these two results in further energy requirements to meet this metabolic cost. Resting energy expenditure increases at the time of pulmonary exacerbations and seems to be related to systemic markers of inflammation. Anorexia is also common in severe disease and with pulmonary exacerbations. Anorexia may be mediated by inflammatory cytokines. This may significantly reduce energy intake and consequently cause weight loss.

There is a well-recognized relationship between nutritional status and lung function and this is likely to be explained by the combination of effects of oxygen costs of breathing and systemic inflammation. Careful management of CF lung disease is

MULTISYSTEM COMPLICATIONS OF CYSTIC FIBROSIS - CAN WE PREVENT?



Preventing multisystem complications in Cystic fibrosis (CF) is difficult. It is critically important to maintain nutrition, stop the damaging effects of diabetes, and improve bone mineral density.

Aggressive treatment of pulmonary infection and inflammation is an important strategy in improving morbidity and reducing mortality in people with CF.



The Chest & Heart Association of Bangladesh

Table 1. Causes of the Malnutrition in Cystic Fibrosis

- Intestinal malabsorption
 - pancreatic enzymes
 - Bicarbonate deficiency
 - Bile salt abnormalities
 - Motility
- Inadequate energy intake
- Increased energy requirements secondary to inflammation, increased work of breathing
- Genetic defect?

therefore a critically important part of nutritional management. Pulmonary exacerbations should be treated promptly and aggressively and disease-modifying therapies such as DNase, azithromycin, and nebulized antibiotics should be optimized to reduce chronic inflammation. DNase therapy and azithromycin have both, in randomized, controlled studies, resulted in small but potentially significant increases in body weight. Appetite-enhancing and anabolic agents have been used to manage undernutrition in CF. The abnormalities in intermediary metabolism result in a net catabolic state and anabolic agent such as insulin, insulin-like growth factor 1, and growth hormone have been shown to have a small impact on weight gain and growth hormone also on growth. Other anabolic agents such as megestrol acetate have also been shown to improve weight gain but have significant side effects, and there are insufficient clinical trials to justify their widespread use.

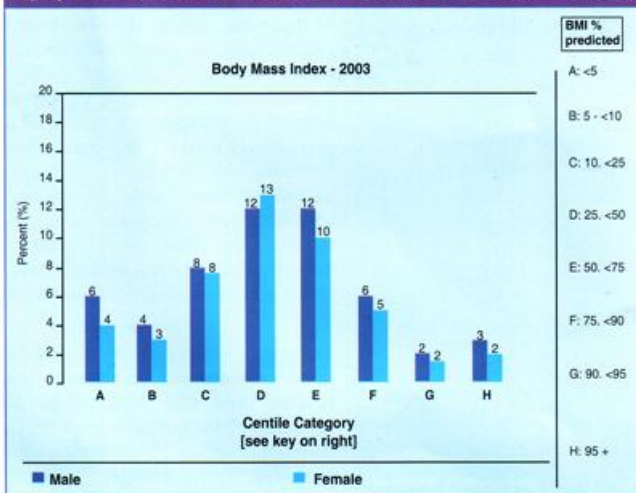
Cystic Fibrosis-Related Bone Disease

CF-related bone disease (CFRBD) is an important systemic complication. CFRBD is very similar to osteoporosis with low bone mass and microarchitectural deterioration of bone tissue. This leads to an increase in bone fragility and

increases the susceptibility to fracture. In general the prevalence of CF-related bone disease is reported as at around 30 to 35% of adults. The prevalence is lower in children and overall the studies would suggest that children should have normal or near normal BMD if lung function and nutrition are maintained. The pathophysiology of low BMD in people with CF has not been entirely determined but there is evidence of increased bone absorption and decreased bone formation. Several factors have been shown to be related to the development of CFRBD. Reduced lung function and pulmonary exacerbations are important factors as are glucocorticoid usage, body mass index, CF-related diabetes mellitus, and CF-related liver disease. Indications of increased inflammation such as C-reactive protein concentrations have been consistently demonstrated as being important factors. Osteoclasts express CFTR, and several recent studies suggest that CFTR dysfunction contributes to the development of CFRBD. Other well-known causes of reduced BMD, such as low participation in exercise and smoking, may also be factors in some individuals.

Several interventions are likely to be very beneficial in preventing the development of CFRBD. Aggressive treatment of lung disease, as already noted, for nutritional interventions is very important. If oral and inhaled glucocorticosteroids must be used, the physician should be careful to titrate to the lowest possible dose because these can have an effect on bone metabolism. Appropriate calcium and vitamin D supplementation is also recommended, though there is no direct evidence to support a protective role in people with CF. Calcium is most effective when supplied as part of the normal diet, and in any case, CF patients should be encouraged to drink milk and other calcium drinks regularly. Vitamin K may also be an important cofactor in maintaining normal BMD. Those with hormonal abnormalities of sex hormones such as low testosterone levels should have these appropriately investigated and treated. Regular physical activity is of benefit in CF for maintenance of general health status. In particular, weightbearing exercise should be encouraged as a useful preventative strategy for CFRBD.

Figure 1: Body mass index (BMI) (% predicted) of children and adults with cystic fibrosis in 2003. Seventeen percent have a BMI <10th centile (groups A and B). The majority are between the 10th and 90th centile and a small number of obese individuals.



Systemic Vasculitis and Cystic Fibrosis Arthropathy

Systemic vasculitis and arthropathy are uncommon complications of CF but can often present considerable challenges in management. Recurrent episodes of distal purpuric vasculitic rash are well described and are probably related again to systemic infection with immune complex deposition in skin capillaries. Autoantibodies to bactericidal permeability-increasing protein (BPI) antibodies has been suggested as important in the pathophysiology of CF arthropathy. This is usually self-limiting, but if troublesome, generally responds to treatment with nonsteroidal anti-inflammatory agents or oral corticosteroids.

Cystic Fibrosis-Related Diabetes Mellitus

CF-related diabetes mellitus (CFRD) is an increasingly common systemic complication of CF. It is rare in children under 10 years but increases with age, occurring in up to 24% of patients aged 20 years and 74% of those over 30 years. The cause of CFRD is multifactorial. Pancreatic fibrosis due to inflammation secondary to obstruction of pancreatic ducts eventually involves pancreatic islet cells. There may also be an intrinsic functional abnormality in CF islet cells. Insulinopenia is a key feature of CFRD and results from the destruction of islet cells. There are, however, other changes in intermediary metabolism that increase insulin resistance. They may be some intrinsic metabolic adaptations that cause this, but systemic inflammation, again, plays a role in increasing insulin resistance.

There are currently no strategies known to prevent the development of CFRD. Reducing lung inflammation will decrease insulin resistance and could be a useful intervention to delay the onset of CFRD. It is important to screen for this condition in all adults with CF. Diabetes has been associated with poorer outcome in people with CF, but it is not clear if correction of the insulinopenia and the successful achievement of good diabetes control alter prognosis. Females with CF seem to be particularly vulnerable to the impact of diabetes, with an even greater impact on prognosis. Treatment of CFRD in general should aim to maintain nutrition with a high-energy diet and matching the insulin required to sustain normal blood glucose control. This is usually best achieved using a combination of long- and short-acting insulin analogs.

Cystic Fibrosis-Related Liver Disease

CF-related liver disease (CFLD) occurs in up to 30% of people with CF. For most there is an abnormality of liver function tests and in some of these, some fatty changes in the liver. In a minority of patients, cirrhosis develops with secondary pulmonary portal hypertension. The pathophysiology of the condition is related to obstruction of small biliary calculi and it is likely that modifier genes in addition to CFTR dysfunction play an important part in this process. There are some studies that have investigated ursodeoxycholic acid as treatment for CF liver disease and some advocate its use to prevent the development of significant cirrhosis. Ursodeoxycholic acid has been demonstrated to be beneficial in primary biliary cirrhosis, however, similar benefits have not been demonstrated in CF-related liver disease. Studies have demonstrated some improvement in liver function tests and one study suggests a reduction in hepatic fibrosis using liver biopsy.

Pancreatitis

Patients with CF and pancreatic sufficiency have an increased risk of developing acute pancreatitis. This is predominantly a problem in individuals with milder CFTR mutations and there has been a suggestion that there may be modifier genes associated with the development of CF-related pancreatitis. No interventions have been shown to prevent such pancreatitis. Idiopathic pancreatitis is associated with CFTR mutations and all such patients should be screened for CF.

Vitamins and Trace Elements

Fat-soluble vitamin deficiency is common in people with CF. Vitamin deficiency rarely causes clinical deficiency syndromes though eye problems with night blindness and conjunctival keratinization have been demonstrated in individuals with low vitamin A concentrations. Neurological complications with peripheral neuropathy have also been demonstrated in vitamin E-deficient patients. Both vitamin A and E have significant antioxidant functions and may be important in the inflammatory response to infection. Vitamin D deficiency is common, particularly in northwestern Europe during the winter months, and may contribute to the development of CF-related bone disease. Vitamin K metabolism is also abnormal in CF and may have several significant consequences if there is a deficiency. This is particularly important in patients with significant liver disease and in those who develop hemoptysis. There is a suggestion that vitamin-deficient patients have more frequent pulmonary exacerbations and lower lung function. Vitamins A, C, and E are important in antioxidant pathways, and deficiency may impair cellular responses to oxidative stress and contribute to cell damage. This may not be a direct cause and effect but it is logical to maintain normal-range vitamin levels in people with CF.

Possible Role of Systemic Antiinflammatory Treatments

Systemic anti-inflammatory therapy with monoclonal antibodies to TNF is now of proven value in rheumatoid arthritis, psoriatic arthritis, and inflammatory bowel disease. These may also be of benefit in refractory asthma. Monoclonal antibodies to tumor necrosis factor- α improve local inflammatory disease and also effectively modulate systemic inflammation. It is possible that anti-tumor necrosis factor treatments or other systemic antiinflammatories will be beneficial in people with CF. It is possible they may improve lung inflammation and so reduce lung injury. They may also be beneficial in stopping the development of CF-related undernutrition and bone disease and in reducing insulin resistance. A recent study in early rheumatoid arthritis demonstrated significant improvement in fat free mass. It is possible that inflammatory fibrosis in the liver and pancreas could be modulated by anti-inflammatory or antifibrotic agents. Better understanding of the inflammatory pathophysiology in CF and its systemic consequences will be important to help identify appropriate drug targets.

Conclusions

Multisystem complications of CF are common. Most of these can be explained on the basis of epithelial cell dysfunction of CFTR or the effect of chronic inflammation or both. Prevention of multisystem complications requires attention to the details of treatment specific to the organ disease and the aggressive treatment of lung disease to ensure that pulmonary and consequently systemic inflammation are kept to a minimum.

[References available on request]



Lung Volume Reduction Surgery Does Not Worsen Pulmonary Hemodynamics

Am J Respir Crit Care Med 2007;176:253-260.

Lung volume reduction surgery (LVRS) for severe emphysema is not associated with increased pulmonary artery pressure, according to recent research findings. "LVRS does not induce the development of pulmonary hypertension in appropriately selected individuals," Dr. Gerard J. Criner from Temple University, Philadelphia, Pennsylvania said. Dr. Criner and colleagues in the National Emphysema Treatment Trial (NETT) research group compared the effect of medical treatment versus LVRS on pulmonary hemodynamics in 110 patients with severe emphysema. Patients who received LVRS had significantly greater FEV₁ predicted, smaller lung volumes, greater diffusion capacity, higher PaO₂, and lower PaCO₂ than did medically treated patients 6 months after randomization, the authors report. Resting end-expiratory pulmonary capillary wedge pressure (PCWP) was also significantly lower in patients who underwent LVRS than in patients treated medically. "Our data show that pulmonary hypertension at rest is not a common complication of bilateral LVRS in our patient group," the investigators conclude. Other hemodynamic values measured during right heart catheterization did not differ significantly between the two groups. "The lack of improvement in resting cardiac parameters post-LVRS, as well as the reduction in end-expiratory PCWP, supports the notion that LVRS improves the functional status in severe emphysema predominantly via its effects on respiratory mechanics," the researchers note. Summing up, Dr. Criner said, "The major benefit from LVRS is an improvement in ventilatory, not cardiac, mechanics." He added: "Complex, large, multicenter, well done trials like NETT can provide important information not only on the primary outcomes but also shed light on the mechanisms of the intervention being tested."

Poor Airway Function in Infancy Predicts COPD in Young Adults

Lancet. 2007;370:717-719, 758-764.

Poor airway function in early infancy should be recognized as a risk factor for chronic obstructive pulmonary disease (COPD) in young adults, according to the results of a nonselective longitudinal cohort study. "Together with smoking, the lung function attained in early adulthood is one of the strongest predictors of chronic obstructive pulmonary disease," write Debra A. Stern, MS, from the University of Arizona in Tucson, and colleagues. "We aimed to investigate whether lung function in early adulthood is, in turn, affected by airway function measured shortly after birth." Between 1980 and 1984, nonselected newborns were enrolled in the Tucson Children's Respiratory Study. At a mean age of 2.3 ± 1.9 months, 169 of these infants underwent measurement of maximal expiratory flows at functional residual capacity (V_{maxFRC}) with use of the chest compression technique. At ages 11, 16, and 22 years, 123 of these participants also had at least 1 measurement of lung function. Outcome measures were forced expiratory volume in 1 second (FEV₁), forced vital capacity (FVC), and forced expiratory flow between 25% and 75% of FVC (FEF₂₅₋₇₅), both before and after treatment with a bronchodilator (180 μ g of albuterol). After adjustments for height, weight, age, and sex, participants with a V_{maxFRC} at infancy in the lowest quartile also had lower values for the FEV₁/FVC ratio (-5.2%; $P < .0001$), FEF₂₅₋₇₅ (-663 mL/second; $P < .0001$), and FEV₁ (-233 mL; $P = .001$) up to age 22 years vs those in the upper 3 quartiles combined. Additional adjustments for wheeze, smoking, atopy, or parental asthma did not alter the magnitude and significance of this effect. "Poor airway function shortly after birth should be recognised as a risk factor for airflow obstruction in young adults," the study authors write. "Prevention of chronic obstructive pulmonary disease might need to start in fetal life." Limitations of the study include lack of measurement of lung function in infancy after bronchodilator use, use of the passive chest-compression technique to obtain partial flow-volume curves, lack of data on static recoil, and a sample too small for accurate determination of the relative contributions of congenital deficits and of acquired factors. The National Heart, Lung, and Blood Institute supported this study. The authors have disclosed no relevant financial relationships. In an accompanying editorial, Michael Silverman, MD, from the University of Leicester, United Kingdom, and Claudia E. Kuehni, MD, from the University of Berne Switzerland, discuss possible fetal causes of impaired lung growth. "Maternal cigarette smoking is the best recognised cause, with independent effects persisting into adult life," Drs. Silverman and Kuehni write. "More work is needed to identify other causes, both environmental (such as nutritional deficiency) and genetic.... As COPD is set globally to become the third most important cause of death, now is the time to add research into its earliest origins to the agenda."



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Snaps

Second-Line More Effective Than First-Line Antibiotics for Acute Exacerbations of Chronic Bronchitis

Chest. 2007;132:447-455.

Second-line antibiotics may be more effective than first-line antibiotics for treatment of acute exacerbations of chronic bronchitis (AECB), according to a meta-analysis of randomized controlled trials. "Traditionally, amoxicillin, ampicillin, doxycycline, or trimethoprim (TMP)/sulfamethoxazole (SMX) have been considered for the treatment of patients with AECBs," write George Dimopoulos, MD, FCCP, from "Attikon" University Hospital in Athens, Greece, and colleagues. "However, given the increasing resistance of *S* [Streptococcus] pneumoniae and *H* [Haemophilus] influenzae to these older antimicrobial agents, the authors of the Canadian guidelines for the management of AECBs stated that therapy with selected second-generation or third-generation cephalosporins or macrolides may be preferable for this patient population. In addition, in light of the recognition that relatively resistant bacterial species, including *P* [Pseudomonas] aeruginosa, may be the etiologic agents of AECBs, especially among patients with significant impairment of lung function, some investigators have advocated the administration of even more potent broad-spectrum antibiotics (eg, quinolones) for the treatment of, at least, this subgroup of CB [chronic bronchitis] patients." The study authors performed a meta-analysis of 12 randomized controlled trials (RCTs) of first-line antibiotics (amoxicillin, ampicillin, pivampicillin, trimethoprim/sulfamethoxazole, and doxycycline) or second-line antibiotics (amoxicillin/clavulanic acid, macrolides, second-generation or third-generation cephalosporins, and quinolones) identified by searching PubMed and the Cochrane databases. In clinically evaluable patients, success of treatment was lower with first-line antibiotics than with second-line antibiotics (odds ratio [OR], 0.51; 95% confidence interval [CI], 0.34 - 0.75). However, first-line and second-line antibiotics were not associated with any significant differences in mortality (OR, 0.64; 95% CI, 0.25 - 1.66) or success of treatment in microbiologically evaluable patients (OR, 0.56; 95% CI, 0.22 - 1.43), or in overall adverse effects (OR, 0.75; 95% CI, 0.39 - 1.45) or diarrhea (OR, 1.58; 95% CI, 0.74 - 3.35). Withdrawals from the study because of adverse effects were also not different between the groups. "Compared to first-line antibiotics, second-line antibiotics are more effective, but not less safe, when administered to patients with AECB," the study authors write. "The available data did not allow for stratified analyses according to the presence of risk factors for poor outcome, such as increased age, impaired lung function, airway obstruction, and frequency of exacerbations; this fact should be taken into consideration when interpreting the findings of this meta-analysis." Other limitations of the study include lack of stratification of patients in the RCTs according to risk factors for poor outcome; grouping of different classes of antimicrobial agents with different in vitro activity; lack of specific data regarding treatment success in intent-to-treat (ITT) patients; inability to evaluate AECB-free interval and time to recovery; lack of data on the eradication of pathogens other than *S* pneumoniae, *H* influenzae, and *Moraxella catarrhalis*; lack of data regarding the emergence of *Clostridium difficile* acquisition in patients receiving antibiotics for AECBs; and different periods in which the included RCTs were conducted. "Second-line antimicrobial agents appear to be more effective than first-line antimicrobial agents for the management of patients with AECBs," the investigators conclude. "This finding provides further support to the suggestion by some experts that advanced antibiotics are preferable to the old antibiotics for this purpose when the administration of antimicrobial agents is recommended."



Diabetes May Impair Tuberculosis Treatment Response

Clin Infect Dis 2007;45:428-435.

Patients with pulmonary tuberculosis and diabetes do not respond as well to TB therapy as those who are non-diabetic, new research indicates.

The reason for this is unclear, but screening for and aggressively treating diabetes may improve the outcomes of patients receiving TB therapy, note Dr. Reinout van Crevel, from Radboud University Medical Center in Nijmegen, the Netherlands in the August 15th issue of Clinical Infectious Diseases.

The findings stem from a study of 737 Indonesian patients with pulmonary TB who were screened for type 2 diabetes and then followed while receiving TB therapy.

Overall, 14.8% of subjects had diabetes, the report indicates. Despite having more symptoms on presentation, patients with diabetes had TB that was comparable in severity to that in non-diabetics.

However, after 2 months of treatment, sputum test results were more likely to be positive in diabetic patients --18.1% vs. 10.0% in non-diabetics based on microscopy. At 6 months, positivity rates of cultured sputum specimens were 22.2% vs. 9.5% (adjusted odds ratio, 7.65; $p = 0.004$).

In a related editorial, Dr. Blanca I. Restrepo, from the University of Texas Health Science Center in Houston, comments that the findings "highlight the need for further research aimed at understanding how the current global epidemic of type 2 diabetes mellitus is affecting TB control and prevention."



Newer Diagnostics for Tuberculosis & MDR-TB

DIAGNOSIS OF TUBERCULOSIS

A major development in the diagnosis of TB was the introduction of several nucleic-acid amplification (NAA) techniques, such as the polymerase chain reaction (PCR) that has been widely evaluated. Several in-house PCR methods have been developed and tested and several studies have been published on their application for TB diagnosis. Initial studies found that lack of specificity was more of a problem than lack of sensitivity not associated with the use of any particular method.

Real-time Polymerase Chain Reaction Techniques

Recently, real-time PCR methods have been proposed for the detection of various microorganisms including *M. tuberculosis*. Real-time PCR methods are based on hybridization of amplified nucleic acids with fluorescent-labelled probes spanning DNA regions of interest and monitored inside thermal cyclers. The fluorescent signal increases in direct proportion to the amount of amplified product inside the reaction tube. This technology has been evaluated in several studies in cultures and more recently in clinical samples. The sensitivity has ranged from 71 to 98% with specificity close to 100%. Due to the irregular distribution of bacilli in the clinical specimens, however, sensitivity of the test could be affected by the initial volume of DNA present.

Non-molecular Techniques

Several new approaches have been recently proposed for the rapid detection of *M. tuberculosis* in clinical samples. Some of them are available as commercial kits while others are in-house approaches. All of them, however, rely on alternative rapid methods of detecting growth other than observation of mycobacterial colonies.

The FastPlaque Tuberculosis Assay. The FastPlaque TB assay is a commercial test based on the technology for the rapid detection of *M. tuberculosis*. It relies on the ability of *M. tuberculosis* to support the growth of an infecting mycobacteriophage. The number of endogenous phages, representing the number of original viable *M. tuberculosis*, is then determined in a rapidly growing mycobacteria such as *M. smegmatis*. Several studies have evaluated this assay in clinical samples. Albert et al. compared the FastPlaque TB assay with auramine smear microscopy and sputum culture on Löwenstein-Jensen medium, detecting TB in 75% of culture-confirmed cases and 70% of cases with a clinical diagnosis of TB with specificities of 98.7 and 99.0% respectively. Auramine smear microscopy, on the other hand, had a sensitivity of 63.4 and 61.3% and specificities of 97.4 and 97.3% in culture-confirmed and all cases respectively. More recently, in a comparative study of the FastPlaque TB test and the original in-house method performed in Zambia, Mbulo et al. found that neither method was able to outperform direct microscopy in sputum samples. Furthermore, 40% contamination was obtained with the FastPlaque TB test, concluding that the phage-based assays offered no advantage for TB diagnosis in that setting. Overall these studies have shown a sensitivity of 50-65% in smear-negative specimens with specificity of 98% and by combining it with smear microscopy the sensitivity reached 80-90% in culture-positive specimens.

Mycobacterial Growth Indicator Tube. Although not really a 'new' diagnostic method since it was proposed almost 10 years ago, the Mycobacterial Growth Indicator Tube (MGIT) is part of the 'new generation' of TB diagnostic tools both in its manual version as well as in its more recently introduced automated format. It is based on fluorescence detection of mycobacterial

growth in a tube containing a modified Middlebrook 7H9 medium together with a fluorescence quenching-based oxygen sensor embedded at the bottom of the tube. Consumption of oxygen in the medium produces fluorescence when illuminated by an ultraviolet lamp. The MGIT system has been thoroughly evaluated in clinical settings for the detection and recovery of mycobacteria. Both the automated and manual MGIT systems have shown similar results that are comparable to those obtained previously with the BACTEC radiometric system. Operational and cost-effectiveness studies assessing their impact in low and middle-income countries are still lacking.

DIAGNOSIS OF TUBERCULOSIS

Early detection of drug resistance in TB allows starting of an appropriate treatment, which has an impact in the better control of the disease. MDRTB constitutes a major threat to TB control. During the last years several new approaches have been proposed to rapidly detect MDRTB including both genotypic and phenotypic methods. In many cases, the genotypic methods especially have been directed to detect rifampicin resistance since it was shown to be a good surrogate marker for MDRTB; more recent evidence, however, suggests that this may not be true for all settings. Genotypic methods have the advantage of a shorter turnaround time, no need for growth of the organism, possibility for direct application in clinical samples, less biohazard risks, and feasibility for automation; not all molecular mechanisms of drug resistance, however, are known. Phenotypic methods are in general simpler to perform and might be closer to being routinely implemented in clinical mycobacteriology laboratories. In the next section these two types of techniques will be addressed.

Genotypic Methods

Genotypic methods look for genetic determinants of resistance rather than the resistance phenotype and involve two basic steps: a molecular nucleic acid amplification such as PCR to amplify sections of the *M. tuberculosis* genome known to be altered in resistant strains and a second step of assessing the amplified products for specific mutations correlating with resistance.

DNA Sequencing. Sequencing DNA of PCR amplified products has been the most widely used method; it is accurate and reliable and it has become the gold standard for mutation detection. It has been performed by manual and automated procedures although the latter is now the most commonly used. It has been widely used for characterizing mutations in the *rpoB* gene in rifampicin-resistant strains and to detect mutations responsible for resistance to other anti-tuberculosis drugs. It would be rather difficult, however, to implement it routinely for detection of drug resistance mutations for several drugs since it would involve several reactions for each isolate, making the cost high.

Solid-phase Hybridization Techniques. There are currently two commercially available solid-phase hybridization techniques: the Line Probe Assay for the detection of rifampicin resistance and the GenoType MTBDR assay for the simultaneous detection of isoniazid and rifampicin resistance. The LiPA assay was introduced several years ago and is based on the hybridization of amplified DNA from cultured strains or clinical samples to 10 probes covering the core region of the *rpoB* gene of *M. tuberculosis*, immobilized on a nitrocellulose strip. The GenoType MTBDR on the other hand, detects

resistance to isoniazid and rifampicin in culture samples based on the detection of the most common mutations in the *katG* and *rpoB* genes respectively. Both assays have now been evaluated in different settings, giving encouraging results.] In a recent study Hillemann et al. evaluated the GenoType MTBDR assay and found that 99% of MDR strains with mutations in the *rpoB* gene and 88.4% of strains with mutations in the codon 315 of the *katG* gene were correctly identified. Correlation with DNA sequencing was 100% and compared with conventional tests good sensitivity and specificity were also obtained. Both solid hybridization methods have shown to be relatively simple to perform although basic expertise in molecular biology and PCR techniques is required. As with other genotypic methods the sensitivity of the test depends on the amount of DNA present in the sample and also the presence of inhibitors could cause false-negative results.

Microarrays. Although technically a solid-phase-type hybridization assay, microarrays, also known as biochips, have been proposed as new molecular methods for detecting drug resistance in *M. tuberculosis*. They are based on the hybridization of DNA obtained from clinical samples to oligonucleotides immobilized in a solid support, such as miniaturized glass slides. They have been mainly used to detect resistance to rifampicin. In a recent evaluation using oligonucleotide microarrays for analysis of drug resistance, Gryadunov et al. detected over 95% rifampicin resistant and almost 80% isoniazid resistant *M. tuberculosis* isolates within 12 h in a sample of drug-resistant isolates and clinical samples. For the time being and due to the high cost involved, the use of microarrays for detecting drug resistance in TB is still beyond the reach of most clinical mycobacteriology laboratories.

Real-time Polymerase Chain Reaction Techniques. Real-time PCR techniques have also been introduced for rapid detection of drug resistance. Different probes have been used like the TaqMan probe, fluorescence resonance energy transfer (FRET) probes, molecular beacons and bioprobes. The main advantages of real-time PCR techniques are the speed of the test and a lower risk of contamination. The main disadvantages would be the requirement for expensive equipment and reagents, and the need for skilled technical personnel. Real-time PCR was initially applied to *M. tuberculosis* strains but more recently it has been successfully applied directly in clinical samples. Results could be obtained in an average of 1.5-2.0 h after DNA extraction. Real-time PCR could eventually be implemented in reference laboratories with the required capacity to properly set up the technique and in settings where it can contribute to the management of TB patients.

Phenotypic Methods

Phenotypic methods assess inhibition of *M. tuberculosis* growth in the presence of antibiotics. During the last years several phenotypic approaches have also been proposed for the rapid detection of drug resistance in TB. As already mentioned, the MGIT system, both in its manual and automated versions, is part of the new-generation of diagnostic techniques for the rapid detection of drug resistance. Many studies have now been published on the application of the MGIT system for rapid detection of resistance to first and second-line anti-TB drugs. In all these studies the MGIT system has shown very good results with a high correlation with conventional methods. The only limitation for a wide implementation of this new technique would be its cost, which can be high in many settings especially in high-endemic countries.

Phage-based Assays. Phage-based assays have also been introduced for the rapid detection of drug resistance in TB.

Both the phage amplification-based tests in its commercial and in-house versions and the luciferase reporter assays have been proposed. The in-house phage amplification test and the FastPlaque TB assay have been mainly tested for the detection of rifampicin resistance both in *M. tuberculosis* isolates and directly on clinical specimens. Luciferase reporter tests have now been evaluated against the four first-line antibiotics with an overall agreement of 98.6% compared with the BACTEC TB-460 (Becton Dickinson, Sparks, Maryland, USA) system. In a recent study Hazbon et al. compared two detection methods, photographic and luminometric, of luciferase reporter phages for susceptibility testing of *M. tuberculosis* against the first-line drugs. The sensitivity for detecting isoniazid and rifampicin resistance was 100%, concluding that both methods were appropriate as screening tests for MDRTB surveillance. The study, however, was performed on *M. tuberculosis* strains. As long as phage-based assays are adapted to be used directly on clinical specimens, they could constitute a good alternative for rapid detection of MDRTB.

Colorimetric Methods

Colorimetric methods are based on the reduction of a coloured redox indicator added to the culture medium after *M. tuberculosis* has been exposed *in vitro* to different antibiotics. Resistance is detected by a change in colour of the indicator, which is directly proportional to the number of viable mycobacteria in the medium. Different indicators have been evaluated for testing against first and second-line drugs, giving comparable results in agreement with the gold standard proportion method. In a multicentre evaluation to assess two colorimetric methods using the MTT and resazurin as redox indicators for the first-line drugs, very good results were obtained with sensitivity and specificity of 99 and 96% respectively. Both tests were easily implemented in the participating laboratories and gave reproducible results. Colorimetric methods seem a good alternative for rapid testing of different anti-TB drugs. These tests in their current format using microtitre plates, however, seem more appropriate for reference laboratories with the necessary biosafety facilities for their manipulation.

The Nitrate Reductase Assay. The nitrate reductase assay (NRA) is a very simple technique based on the capacity of *M. tuberculosis* to reduce nitrate to nitrite, which is detected by adding a chemical reagent to the culture medium. *M. tuberculosis* is cultivated in the presence of an antibiotic and its ability to reduce nitrate is measured after 10 days of incubation. Resistant strains will reduce the nitrate, revealed by a pink-red colour in the medium, while susceptible strains will lose this capacity as they are inhibited by the antibiotic. The test was recently evaluated for first and second-line drugs with good results. It has the added advantage of using the same format and culture medium as used in the standard proportion method. A recent multicentre study evaluated the performance of the NRA to detect resistance to the first-line drugs. The test performed very well for isoniazid, rifampicin and ethambutol with accuracy between 96.6 and 98%. Lower values, however, were obtained for streptomycin. The NRA was easily implemented in countries with limited laboratory facilities. The main advantage of the NRA, in addition to its simplicity, is that it uses the same format and culture medium as used in the conventional method, facilitating its implementation in diagnostic laboratories.

[References available on request]

Association News

The 28th Annual General Meeting & Scientific conference of The Chest & Heart Association of Bangladesh was held at BCPS Auditorium on March 22, 2007 at 9-30AM. Major Gen.(Rtd) ASM Matiur Rahman, Hon'ble Adviser, Ministry of Health & Family Welfare grace the occasion as Chief Guest, and Dr. Md. Shahjahan Biswas, Director General of Health Services was present as Special Guest. President of the association Professor Mirza Mohammad Hiron presided the inaugural session. Session was started after recitation from Holy Quran. After that welcome address was given by Prof. Mustafizur Rahman, Vice President of the association & Director, NIDCH. Activity report was presented by Dr. Zahirul Islam Shakil followed by Speech by Special Guest, Chief Guest, President of the association, Vote of Thanks by Dr. Rafiqul Islam, Treasurer of the association. At 11.00-12.00 Scientific Session-1 started, six papers were presented respectively by Dr. Rajashish Chakraborty, Dr. AKM Mostafa Hossain, Prof. GM Akbor Chowdhury, Dr. Md. Wahiduzzaman Bhuiyan, Dr. Golam Sarwar LH Bhuiyan & Dr. Sayedul Islam. Session was Chaired by Prof. AKM Shamsul Huq and Prof. Shafiqul



Ahsan & Dr. Md. Abdul Qayyum act as Rapporteur. In Scientific Session-2 six papers were presented by Dr. Shamim Ahmed, Dr. AKM Akramul Haque, Dr. Md. Azizul Haque, Dr. Md. Kamrul Alam, Dr. Manabendra Biswas and Dr. Ahmed Sarwar Murshed. Session was Chaired by Prof. Md. Mustafizur Rahman and Prof. GM Akbor Chowdhury & Dr. MA Rouf was present as Rapporteur. In Scientific Session-3, Cardiology Session, six papers were presented by Dr. Dipanker Chandra Nag, Dr. Mohammad Shafiqur Rahman Patwary, Dr. Md. Golam Kibria, Dr. Md. Tawfiqur Rahman, Dr. Md. Saleh Uddin, Dr. SM Mostafa Zaman. This session was chaired by Prof. Mirza Mohammad Hiron & Prof. Sohely Rahman & Repporteur was Dr. Bazlul Gani Bhuiya. At 4:00PM Annual General Meeting was started with Professor Mirza Mohammad Hiron in the Chair. Annual Report was presented by Dr. Zahirul Islam Shakil, Audit Report by Treasurer Dr. Md. Rafiqul Islam. After brief discussion meeting was concluded after address of President. Sight Seeing program was held to Cox's Bazar & Banderban from 23rd to 26th March, 2007. Total program was sponsored by SANDOZ.

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