

Synopses of Research Articles

Injections and HIV in Rural Zimbabwe

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Of the 40 million people worldwide with HIV, 30 million live in the developing world. By far the worst hit region is sub-Saharan Africa, where nearly four million children have lost one or both parents to HIV/AIDS since 2000. Is heterosexual transmission the driving force behind the HIV epidemic in sub-Saharan Africa? In a controversial debate, some researchers have suggested that other factors such as unsafe medical injection practices may also be to blame, and that by overlooking, and even suppressing, analysis of this possible route of transmission, the current focus on preventing sexual transmission may be misguided. In this month's *PLoS Medicine*, Ben Lopman and colleagues argue that although it is right to criticize the lack of evidence on unsafe medical injection, field data are hard to collect. They note that in the only published study addressing this issue, Kiwanuka and colleagues found no link between unsafe injections and HIV spread in rural Uganda. In an effort to "inform the debate" further, Lopman and colleagues looked at the association between HIV and unsafe injection practices in rural Zimbabwe.

The team analyzed data from adults in Manicaland, a rural part of Zimbabwe, who were taking part in the Manicaland HIV/STD Prevention Study. In 1999 and 2000, eligible patients were tested for HIV and surveyed (86.7% were HIV negative at the start of the study), and were followed up three years later.

The team collected survey data on injections in the patients, who were male and female adults aged 15 to 54 years old, and tested for an association between injection exposure and HIV infection.



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Are medical injections an important cause of HIV in rural Africa?

In 2002 and 2003, 505 of the men and 1,342 of the women, representing a 69.7% follow-up, were again interviewed and tested for HIV infection. Of these, 40% reported having had an injection or needle prick during the study period. A total of 67 patients developed HIV

during the study; of these 13 (19%) said they had not had sex during the study period and 40 (60%) said they had not had an injection. The statistical analysis found no significant association between injections and HIV infection in men or women.

Patients who had HIV when the study began did not have higher rates of injections. Instead, injections were highly associated with childbirth and pregnancy. But since HIV-positive women have reduced fertility, a reduction in the use of maternal services may partially explain why injections were not more common in these HIV-positive patients. In this study, the strongest predictor of HIV infection was symptoms of sexually transmitted disease.

Despite problems of recall bias and under-reporting of sexual activity—a particularly difficult problem in studies in Africa—sexual behavior is consistently linked with HIV incidence. Where does this leave the debate over injections in Africa? Certainly, for this community, they do not seem to be a major source of HIV infection, and local policy-makers would therefore do best to concentrate on the prevention of sexually transmitted infections.

Lopman BA, Garnett GP, Mason PR, Gregson S (2005) Individual level injection history: A lack of association with HIV incidence in rural Zimbabwe. DOI: 10.1371/journal.pmed.0020037

Mass Spectrometry–Based SARS Genotyping

DOI: 10.1371/journal.pmed.0020052

To quickly control infectious disease outbreaks, extensive information is required to identify the source and transmission routes, and to evaluate the effect of containment policies. Traditionally, scientists have used travel- and contact-tracing methods, but the recent SARS epidemic showed that sequence-based techniques for pathogen detection can also be important tools to help understand outbreaks. Jianjun Liu and colleagues adapted mass spectrometry (MS)–based genotyping, already used as a high-throughput way of detecting single nucleotide polymorphisms in human DNA, to the analysis of the SARS virus from clinical samples.

The major breakthroughs against SARS were the discovery of the SARS coronavirus (SARS-CoV) as the etiological agent

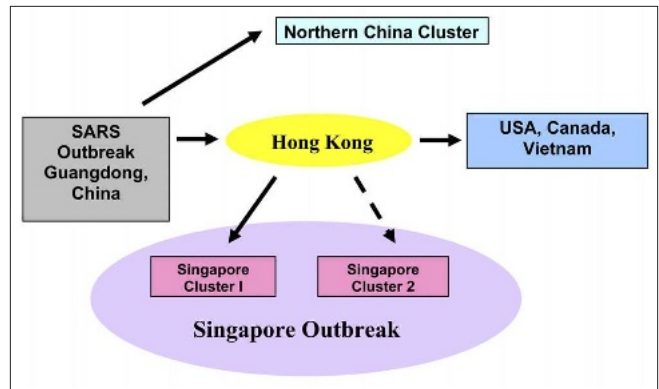
and the sequencing of the SARS genome. Liu's colleagues at the Genome Institute of Singapore had previously shown that common genetic variants in the SARS-CoV genome could be used as molecular fingerprints to help trace the route of infection. However, as "sequence analysis of large numbers of clinical samples is challenging, cumbersome, and expensive," they felt that "what is needed is a rapid, sensitive, high throughput, and cost-effective screening method." Towards this goal, Liu and colleagues now demonstrate that an MS-based technique can quickly yield accurate information on clinical isolates (in this case from the 2003 SARS outbreak in Singapore).

The scientists demonstrate the sensitivity of the assay in detecting SARS-CoV variations and test it further in cultured viral

isolates and uncultured lung tissue samples of SARS-CoV. They analyzed isolates taken from 13 patients with SARS at different stages of the Singapore outbreak, identified nine sequence variations, and discovered a new primary route of introduction of the virus into the Singapore population. They also found a Singaporean origin for a German case of SARS, a result that could not be derived from standard sequencing methods. The analysis of the uncultured lung tissue also found different sequences in a single patient, which suggested the presence of multiple viral sequence variants in one host.

The study suggests that MS-based genotyping can be used for large-scale genetic characterization of viral DNA from clinical samples. The researchers found that the method was accurate and sensitive, with a 95% success rate for detecting sequence variations at low virus concentrations. The MS-based assay allows high-throughput analysis and complements the “gold standard” direct sequence analysis method, which is used to identify new sequence variations. As such, it is particularly useful for investigating agents for which extensive sequence information exists.

Liu and colleagues propose that the most efficient method for a large-scale population investigation would be initial characterization of a genome sequence by direct sequence analysis in a subset of samples, followed by MS-based analysis of



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Two transmission routes for the Singapore SARS outbreak

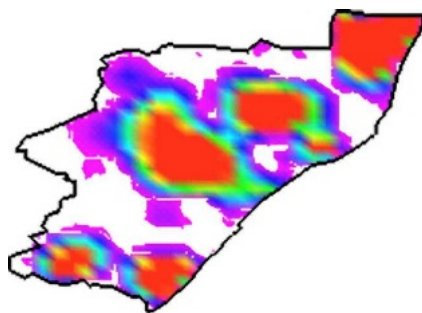
informative genetic variations. Altogether, their results suggest that MS-based genetic analysis can help real-time investigations in disease outbreaks.

Liu J, Lim SL, Ruan Y, Ling AE, Ng LFP, et al. (2005) SARS transmission pattern in Singapore reassessed by viral sequence variation analysis. DOI: 10.1371/journal.pmed.0020043

Equitable Allocation of Antiretrovirals in Resource-Constrained Countries

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Antiretroviral drugs change the lives of patients with HIV/AIDS—if they have access to them. Most patients in resource-poor countries cannot afford the drugs. Major initiatives are under way to expand access to antiretrovirals in developing countries, but the number of individuals in need of the drugs currently vastly exceeds the supply, and will continue to do so for the foreseeable future. These circumstances make for difficult decisions about treatment allocation. David Wilson and Sally Blower have shown how it is possible to design an equitable antiretroviral allocation strategy, that is, to come up with a plan that would give each individual with HIV an equal chance of receiving antiretrovirals. Their novel spatial model enables them to model the “spatial



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Modeling allocation of antiretrovirals in KwaZulu-Natal

diffusion” of antiretrovirals in a resource-constrained country.

Based on the premise that only a limited number of drugs will be available and only a limited number of health-care facilities can be used for drug distribution (each of them serving the population in a specific area), they determine an optimal equitable allocation strategy. They then apply this approach to a practical example—the equitable allocation of antiretrovirals to patients with HIV/AIDS in the South African province of KwaZulu-Natal. Using data from a detailed rollout plan for antiretrovirals designed by the South African government, they come up with an allocation strategy that differs substantially from the current governmental plan for the province.

KwaZulu-Natal has a total of 54 health-care facilities, of which 17 are assigned to allocate antiretrovirals under the current plan. It is the largest province in South Africa, with a population of about 9.4 million, and it has more people with HIV than any other province (about 21% of all cases in South Africa). Wilson and Blower assume that the available amount of antiretrovirals can treat 10% of the individuals with HIV in KwaZulu-Natal. Modeling the 17 health-care facilities and the 51 communities of individuals with HIV, they determine the amount of drugs to allocate to each facility to achieve equitable access by patients throughout the province. They then extend the

analysis assuming that additional health-care facilities could be made available to distribute drugs. They conclude that in order to achieve the greatest degree of treatment equality, all 54 health-care facilities should be used, and they should, on average, each serve the population within a radius of 50 km.

Wilson and Blower discuss how their model can be adjusted and therefore used by policy makers in resource-constrained countries to determine a scientifically based allocation strategy for limited resources based on a number of specific objectives. They also recognize that there are other considerations that influence ethical treatment allocation besides equity, for example, the desire to maximize epidemic reduction, or the imperative to give priority to the least advantaged individuals. They believe that their model can be adjusted and therefore “used by policy makers to determine an optimal scientifically based allocation strategy” for a number of specific objectives. Another possibility would be to apply the equity strategy to allocate drugs to particular health-care facilities (thereby achieving equality in accessibility), and then take additional ethical considerations into account at the community level.

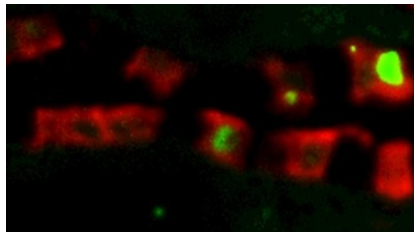
Wilson DP, Blower SM (2005) Designing equitable antiretroviral allocation strategies in resource-constrained countries. DOI: 10.1371/journal.pmed.0020050

Why Blood Glucose Control Matters for the Kidney

DOI: 10.1371/journal.pmed.0020056

One of the most common and most serious complications of both type 1 and type 2 diabetes is diabetic nephropathy. It occurs in around 30% of patients with type 1 diabetes and 10% to 40% of patients with type 2 diabetes. Diabetic nephropathy is the leading cause of renal failure in the developed world. The main effect of diabetic nephropathy is proteinuria, initially in very small amounts but which increases, leading to nephrotic syndrome and end-stage renal disease in most cases.

Various risk factors in individuals with diabetes are known to increase the chance of developing diabetic nephropathy, including South Asian or African background, male sex, long history of diabetes, poor blood sugar control, high blood pressure, and smoking. One early change associated with diabetic nephropathy is degeneration of the renal tubular epithelium, but the exact cause of this at the cellular level is unclear. Erwin Böttinger and colleagues have dissected out one key point in the progression to diabetic nephropathy. They looked at cell lines of renal tubular cells from humans and mice and kidney biopsies



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Apoptotic renal tubular cells in diabetic nephropathy

from patients with diabetic nephropathy, patients with non-diabetic renal disease, and mice with genetic and induced diabetes. In the human cell lines they showed that glucose induced the expression of CD36, a receptor known to have a role in adhesion and signal transduction (in addition to being the receptor for malaria-infected erythrocytes). They then went on to show that apoptosis of these cells occurred in the presence of glycated (glucose-modified) albumins or free fatty acids, which are present in increased amounts in patients with diabetes, and that CD36 was essential for the apoptosis to occur. They then examined how CD36 triggered

apoptosis and found that it involved src kinase, p38 MAP kinase, and caspase 3.

Comparing mice and humans, the researchers found that the two species are not alike: diabetic mice did not show an increase in tubular expression of CD36—even though the gene is present in mice—and had normal tubular epithelium and no tubular apoptosis. They confirmed this difference between humans and mice by showing that normal mouse epithelial cell lines were resistant to apoptosis caused by the glycated albumins; however, artificially expressing CD36 in these lines made them susceptible to apoptosis by these modified albumins.

These results provide insight into one of the crucial steps in diabetic nephropathy and, in humans at least, might help to explain why high blood glucose is so damaging to the kidney, hence providing a good reason—if another is needed—for encouraging patients to control blood glucose as tightly as possible.

Susztak K, Ciccone E, McCue P, Sharma K, Böttinger EP (2005) Multiple metabolic hits converge on CD36 as novel mediator of tubular epithelial apoptosis in diabetic nephropathy. DOI: 10.1371/journal.pmed.0020045

Towards Better Evaluation of Pneumococcal Vaccines

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Pneumonia remains the leading cause of death worldwide in children. Several vaccines against pneumococcal pneumonia are at various stages of development, but the testing of their efficacy is hampered by the lack of noninvasive tests that are sensitive and specific for the disease. Diagnosis is usually based on chest radiographs, which are not very specific for pneumococcal disease.

In their quest for a more specific diagnostic test, Shabir Madhi and colleagues—who are conducting clinical trials on pneumococcal vaccines in children—examined whether serum concentrations of procalcitonin and C-reactive protein could improve the specificity of chest radiographs to diagnose pneumococcal pneumonia and thus be useful in the future evaluation of pneumococcal vaccines. Elevated levels of both proteins are associated with bacterial disease. They might therefore help to differentiate bacterial from nonbacterial causes of pneumonia, and thus allow to “enrich” the analyzed disease cases for those of pneumococcal origin, against which the vaccine is potentially active.

This study represents a first step, in which the researchers tested whether adding information about procalcitonin and C-reactive protein levels to data from a completed vaccine trial would affect the outcome regarding vaccine efficacy. When re-analyzing previous trial data under these conditions, the vaccine appeared more efficacious compared with placebo when either

elevated procalcitonin or elevated C-reactive protein levels were taken into account. The efficacy estimate was greatest when cases of pneumonia that had elevated levels of both procalcitonin and C-reactive protein were compared against placebo.

These data suggest that elevated levels of C-reactive protein and procalcitonin, in conjunction with chest radiography, could improve the specificity of a diagnosis of pneumococcal pneumonia over that of chest radiography alone. This combined diagnostic test could be useful for further evaluation of pneumococcal vaccines. The hope is that among patients identified as having pneumonia by the combined test, a higher proportion would have pneumonia of pneumococcal origin. As a consequence, there would be less “background noise” caused by other forms of pneumonia, and this should make it easier to assess the efficacy of vaccine candidates. However, as the researchers point out, this analysis was not a primary objective of the present trial. This analysis can therefore serve only as a hypothesis-generating study, and as such the hypothesis must be tested in other study settings.

The study was sponsored by Wyeth, manufacturers of the pneumococcal vaccine used.

Madhi SA, Heera JR, Kuwanda L, Klugman KP (2005) Use of procalcitonin and C-reactive protein to evaluate vaccine efficacy against pneumonia. DOI: 10.1371/journal.pmed.0020038