Synopses of Research Articles

How Do Nigeria's Health-Care Personnel Treat Patients with HIV/AIDS?

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People living with HIV/AIDS (PLWA) face many forms of stigma and discrimination. This is the case in whichever country they may live, as has been shown in a number of previous research studies. In addition to experiencing unfair treatment in their families, communities, and places of work, PLWA may encounter discrimination from health-care professionals. This can interfere with effective prevention and treatment. Discriminatory practices in the health-care sector may also appear to legitimize other forms of discrimination against PLWA.

Vincent Iacopino and colleagues from the organization Physicians for Human Rights, in collaboration with researchers from Policy Project–Nigeria and the Center for the Right to Health (also in Nigeria) investigated the problem in Nigeria. With a population of roughly 130 million, Nigeria is home to one in 11 of the 40 million PLWA worldwide. Around 6% of adult Nigerians are thought to be HIV-positive, and there will be an estimated 310,000 AIDS deaths this year. The indications are that infection rates will increase. Until now, little has been known about the nature and extent of discrimination against patients with HIV/AIDS in Nigeria.

Trained interviewers conducted a cross-sectional questionnaire survey of 1,021 Nigerian health-care professionals in 111 health-care facilities in four of Nigeria's 36 states. Those sampled were 324 physicians, 541 nurses, and 133 midwives, and 23 health-care workers of unknown profession. Fifty-four percent of them worked in public tertiary care facilities. Many of the survey's results are worrying. Nine percent of professionals reported refusing to care for a patient with HIV/AIDS, and 9% said they had refused a patient with HIV/AIDS admission to a hospital. Fifty-nine percent agreed that PLWA should be on a separate ward, and 40% believed a person's HIV status could be determined by their appearance. Ninety-one percent agreed that staff should be informed when a patient was HIV-positive in order to protect themselves. Forty percent believed health-care professionals with HIV/AIDS should not be allowed to work in any area of health-care requiring patient contact. Twenty percent agreed that many with HIV/AIDS had behaved immorally and deserved their infection. Eight percent felt that treating someone with HIV/AIDS was a waste of resources.

Providers who reported working in facilities that did not always practice universal precautions against HIV transmission were more likely to favor restrictive policies towards PLWA. In general, basic materials needed for treatment and prevention of HIV infection were not sufficiently available. Providers who reported less adequate training in HIV/AIDS treatment and in ethics were more likely to report negative attitudes towards patients with HIV/AIDS. There was no consistent pattern of differences in negative attitudes and practices across the different professions surveyed.

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Training of interviewers for the study included 5 days of classroom teaching and role-playing

The researchers concluded that, while most health-care professionals surveyed reported being in compliance with their ethical obligations, discriminatory behavior and attitudes towards patients with HIV/AIDS existed among a significant proportion. Inadequate education about HIV/AIDS and a lack of protective and treatment materials appear to favor these practices and attitudes. The findings of the study, in just four states, cannot be generalized to Nigeria as a whole and, although sampled systematically, it is possible that sampled facilities and health-care professionals may differ significantly from those that were not sampled in the study states. Concerns over a perceived lack of privacy in the interviews or about job status may have resulted in an underreporting of discriminatory behavior and/or an overreporting of "correct" practices or attitudes. The authors note that the health-care system in Nigeria is underfunded and suffers from fundamental problems, including material scarcity and inadequacies in infrastructure, both of which may contribute to discriminatory behavior. They call for targeted education of health-care professionals and provision of adequate resources to health-care facilities, and for the introduction and enforcement of anti-discrimination policies.

Reis C, Amowitz LL, Heisler M, Moreland RS, Mafeni JO, et al. (2005) Discriminatory attitudes and practices by health workers toward patients with HIV/AIDS in Nigeria. DOI: 10.1371/journal.pmed.0020246

A Novel Virus for Croup

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The World Health Organization estimates that about 20% of all deaths in children younger than five years old are due to acute lower respiratory tract infections (LRTIs), with 90% of these deaths due to pneumonia. But despite LRTIs being among the most frequent diseases in the first years of life, the viral causes of these illnesses are not always clear. Several viruses are known to be involved, e.g., respiratory syncytial virus (RSV), influenza viruses, parainfluenza viruses, and human metapneumovirus, but none of these pathogens is detected in a substantial number of cases.

In a new article published in *PLoS Medicine*, Lia van der Hoek and colleagues investigate the association of acute LRTIs with human coronavirus HCoV-NL63, a virus they recently described. They suggest that HCoV-NL63, a new member of the Coronaviridae family, is one of the most frequently detected viruses in children less than three years old with LRTIs and that this virus is strongly associated with croup.

The team analyzed samples from the PRI.DE study, a prospective population-based study of LRTIs in children younger than three years old in Germany. They assessed by PCR 949 samples of nasopharyngeal secretions from children with LRTIs.

In all, 49 samples (5.2%) were positive for HCoV-NL63 RNA. Viral RNA was more prevalent in samples from outpatients (7.9%) than hospitalized patients (3.2%), and co-infection with either RSV or parainfluenza virus 3 was observed frequently. With an overall occurrence of 5.2%, HCoV-NL63 was the third most frequently detected pathogen in this patient group (RSV was the highest, found in 31.4% of samples). The researchers focused on HCoV-NL63 in cases of respiratory disease where no other viral pathogen could be detected in order to identify clinical symptoms associated with HCoV-NL63 infection. Samples in which only HCoV-NL63 RNA could be detected had a significantly higher viral load than samples containing additional respiratory viruses. A strong association with croup was apparent: 43% of the HCoV-NL63-positive patients with high HCoV-NL63 load and absence of co-infection had croup, compared with 6% in the HCoV-NL63-negative group. Indeed, a significantly higher fraction (17.4%) of samples from croup patients than non-croup patients (4.2%) contained HCoV-NL63 RNA.

This study strengthens the evidence for the role of HCoV-NL63 in croup. Previous studies have shown croup to occur mostly in boys, with peak occurrence in the second year of life and predominantly in the late autumn or early winter season. And HCoV-NL63 infection seems to follow these trends, said the authors.

However, the authors warned that the high percentage of HCoV-NL63-positive samples could be due to a strong viral activity in the study year, and long-term studies are needed to determine whether HCoV-NL63 infections occur in cycles peaking every two to three years, as observed for other respiratory viruses. But they noted that HCoV-NL63 has spread worldwide, with the virus found in Australia, Canada, Japan, Belgium, and the US. Thus, health authorities should add HCoV-NL63 to the list of pathogens that can cause numerous LRTIs in young children.

van der Hoek L, Sure K, Ihorst G, Stang A, Pyrc K, et al. (2005) Croup is associated with the novel coronavirus NL63. DOI: 10.1371/journal.pmed.0020240

Measuring Hidden Parasites in Falciparum Malaria

DOI: 10.1371/journal.pmed.0020268

Approximately 40% of the world's population, mostly living in the world's poorest countries, is at risk of malaria. In the tropical and subtropical regions of the world, malaria causes 300 million acute illnesses and at least 1 million deaths annually. Ninety percent of these deaths occur in Africa, south of the Sahara, mostly among young children.

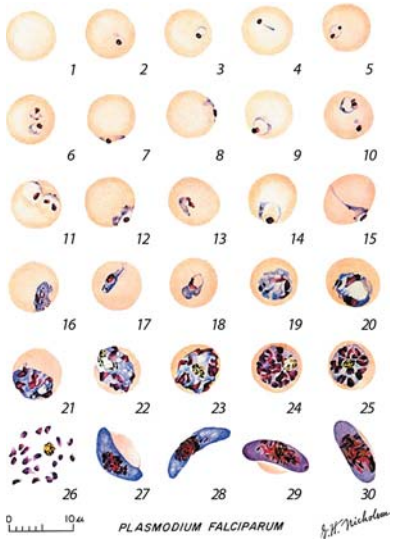
To assess disease severity, peripheral blood parasitemia is measured, but this is only a weak predictor of mortality in falciparum malaria. In addition, a microscopist is only able to count the less pathogenic circulating stages of the parasite, whereas the more pathogenic parasitized erythrocytes, sequestered in the capillaries and containing mature parasites, are not seen and therefore not counted. However, sequestered *Plasmodium falciparum* parasites secrete Histidine-rich protein 2 (PfHRP2), which is liberated into the plasma at schizont rupture.

In this month's *PLoS Medicine*, Arjen Dondorp and colleagues suggest that the plasma concentration of this protein might provide a better estimate for the patient's total parasite biomass and therefore be a more accurate prognostic indicator than circulating parasite load. There is evidence to support this hypothesis. A recent study by the same team measured PfHRP2 in *P. falciparum* cultures, and showed that approximately 89% of PfHRP2 is liberated at schizont rupture and that the variation in the amount released is limited.

In the current study the researchers measured plasma PfHRP2 concentrations in 337 patients with varying severity of falciparum malaria and, using a simple mathematical model, estimated the total body parasite biomass. This value was compared with measures of disease severity and outcome. The developmental stage distribution of circulating parasites, which also provides information on the sequestered parasites, was also evaluated in relation to plasma PfHRP2 levels in these patients.

The researchers found that the estimated geometric mean parasite burden was more than six times higher in patients with severe malaria than in patients hospitalized without signs of severe disease, and was highest in patients who died. Statistical analysis revealed that the estimated total parasite biomass was clearly associated with disease severity and outcome. By contrast, peripheral blood parasitemia and the number of circulating parasites were not associated with disease outcome, nor with other measures of severity such as admission plasma lactate concentrations.

The finding that sequestered parasite biomass is associated with disease severity fits with current thinking that sequestration of erythrocytes containing the mature forms of the parasite is the central pathological process in falciparum malaria.



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Stages in the life cycle of *Plasmodium falciparum*
(Illustration: Coatney GR, Collins WE, Warren M, Contacos PG (1971) The primate malarias. (Bethesda): U.S. Department of Health, Education and Welfare.)

However, the team noted there were several factors that might contribute to inaccuracies in the model. For example, the amount of PfHRP2 secreted per parasite varies between different parasite strains. Also, in high transmission areas, where partial immunity against the disease develops,

clearance of PfHRP2 might be increased in the presence of antibodies against the protein; in these areas—such as countries in sub-Saharan Africa—the model would thus underestimate the parasite burden and might need to be adapted further for use.

Despite these issues, estimates of plasma PfHRP2 concentrations may be useful as a research tool to stratify patients' parasite loads, say the authors. They conclude that quantitative measurements of plasma PfHRP2 in patients with falciparum malaria could be used to estimate the total parasite biomass, a parameter pivotal in the pathophysiology of the disease, and that this total parasite biomass is associated with clinical measures of the severity of the disease.

Dondorp AM, Desakorn V, Pongtavornpinyo W, Sahassananda D, Silamut K, et al. (2005) Estimation of the total parasite biomass in acute falciparum malaria from plasma PfHRP2. DOI: 10.1371/journal.pmed.0020204

Modeling HIV Vaccine Strategy in Animals

DOI: 10.1371/journal.pmed.0020258

Animal models can play an essential role in guiding preclinical vaccine development, including in studies of preclinical vaccine safety, vaccine toxicity, and vaccine immunogenicity. Appropriate pathogen challenge models can also provide the opportunity to perform preclinical tests of vaccine efficacy. Preclinical tests of HIV vaccine efficacy are usually performed by exposing macaques to simian immunodeficiency virus (SIV), a virus that is closely related to HIV. However, the viral inoculum sizes used to infect macaques with SIV vastly exceed the amounts of HIV that humans are exposed to during a given exposure. Typically animals are exposed to 10–100 times the infectious dose at which 50% of the animals become infected (ID50). These excessive doses may not provide realistic preclinical tests of vaccine efficacy. Indeed, no vaccine has been shown to be effective in preventing infection by SIV (so-called sterilizing immunity) in such high viral inoculum trials. Now, in a paper published in *PLoS Medicine*, Roland Regoes and colleagues speculate that an alternative approach to trials in animals not only can mimic the human patterns of repeated low-dose exposure, but also can remove one concern for animal researchers—the need to use very large numbers of animals in experiments.

What the researchers did was use statistical power analysis to compare a single low-dose challenge design, in which each animal is challenged only once, and a repeated low-dose challenge design, in which each animal is challenged until it is infected or a predetermined maximum number of challenges is reached. The statistical power of an experimental design—a measure of the statistical quality—was assessed by simulating experiments, evaluating them, and then repeating the procedure thousands of times.

What they found was that the experimental design using a single low dose of virus in each animal required unfeasibly large numbers of animals; even for the highest modeled vaccine efficacy of 90% the single low-dose challenge design required more than 20 animals per group to reach a statistical power of 95%. However, when the researchers modeled a protocol of repeatedly challenging the (virtual) animals with a challenge dose of one ID_{50}, and allowing for a maximum number of 20 challenges of each individual animal, as few as five animals were required to achieve more than 95% of statistical power.

Where do these results leave the design of HIV trials? To begin with, the results should encourage researchers to develop animal models that reflect, to the fullest extent possible, what is known about the natural history and pathogenesis of the disease in humans, rather than designing trials to fit the animal models that are available. The authors have made available the programming script of their analysis so anyone can repeat it; it would be interesting to know whether preclinical trials assessing vaccines or treatments against infections by other pathogens could be usefully modeled in this way as well.

Regoes RR, Longini IM Jr, Feinberg MB, Staprans SI (2005) Preclinical assessment of HIV vaccines and microbicides by repeated low-dose virus challenges. DOI: 10.1371/journal.pmed.0020249

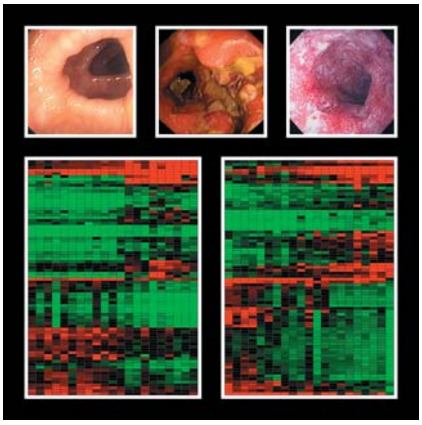
Dissecting Out Differences in the Transcriptomes of Inflammatory Bowel Disease

DOI: 10.1371/journal.pmed.0020273

Inflammatory bowel disease (IBD) is the term that encompasses chronic relapsing diseases characterized by inflammation of the bowel, specifically ulcerative colitis (UC) and Crohn disease (CD). They are common disorders; in the UK, for example, together they affect about one person in every 400, amounting to a total of 120,000 cases of UC and 60,000 of CD, with 6,000 new cases of UC and 3,000 new cases of CD every year. Current estimates total at more than 1 million cases in the US and Europe. The symptoms for both these conditions—which suggest an abnormal immune response at the intestinal mucosa—include abdominal pain, diarrhea, fever, severe fatigue, and weight loss. Current understanding of disease pathogenesis suggests a complex action of multiple environmental factors that trigger disease in individuals with a susceptible genetic background.

Today, finding genes that have a role in diseases has been made easier by the sequencing of the human genome and creation of an expressed sequence tag clone database. Previous positional cloning studies have revealed three genes associated with IBD that carry variants with a causative role. Another of the tools for analyzing genes is microarray technology, in which the expression of transcripts of thousands of genes can be investigated simultaneously. This approach offers an insight into disease pathophysiology.

In a research article published in *PLoS Medicine*, Christine Costello and colleagues have gone further with cDNA microarrays to attempt to decipher gene regulatory events and identify genes that might be involved in the pathophysiology of these IBD. They found 650 genes that were differentially regulated between normal control individuals and the individuals with one of the two IBD subtypes. In fact, 500 and 272 differentially regulated transcripts were identified between control individuals and patients with CD and UC, respectively. There was an imbalance between over- and underexpressed genes in the IBD subtypes. In CD, approximately 84% of differentially expressed genes were found to be down-



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Colonoscopy images of normal control, Crohn Disease (CD) and ulcerative colitis (UC) patients (top, left to right). Differentially expressed genes in controls vs. CD (lower left) and controls vs. UC patients (lower right).

regulated compared with 42% of genes in UC. However, the authors caution that this finding was highly influenced by the types and numbers of genes present on any microarray system; in addition, none of the 122 differentially expressed genes in CD and UC was overexpressed in one disease and underexpressed in the other. These observations support the notion of a shared general inflammatory profile underlying each form of IBD, with more specific events in the pathophysiological cascade being disease-specific.

In a second part of the study, the team tried to interpret the functional consequences of changes in gene expression observed in the microarray analysis. They used an annotation-based pathway database, which classified differentially expressed genes into three major groups: immune and inflammatory response; oncogenesis, cell proliferation, and growth; or structure and permeability. For immune and inflammatory response, the team identified many genes associated with aberrant immune response; it is not surprising, perhaps, to find a general up-regulation of immune response and antigen presentation in IBD. Several genes associated with cell growth and proliferation were up-regulated in UC—a finding similar to previous microarray studies that had reported involvement of cancer-related genes in IBD (although the altered genes were different). There was also an enrichment of genes associated with structure and permeability; in this class several genes were ubiquitously altered in both IBD and non-IBD samples, reflecting dysregulation of genes for paracellular permeability, degradation of extracellular matrix, and barrier protection against bacterial invasion of the epithelial surface.

Ultimately this study highlights the complex pathogenesis of UC and CD, and indicates some possible future avenues for research of mucosal diseases in general. It demonstrates that genomic technologies are suitable to directly dissect human pathophysiology.

Costello CM, Mah N, Häsler R, Rosenstiel P, Waetzig GH, et al. (2005) Dissection of the inflammatory bowel disease transcrptome using genome-wide cDNA microarrays. DOI: 10.1371/journal.pmed.0020199

UK Dementia Incidence Doesn't Vary across Sites with Known Variation in Vascular Risk

DOI: 10.1371/journal.pmed.0020275

Dementia remains an incurable condition and its increasing prevalence is a deeply worrying aspect of the "graying" of the population. An important question for researchers is to establish whether dementia incidence, prevalence, and national history vary from one location to another. Incidence studies are particularly valuable for less biased comparison of disease occurrence, as well as being essential for policy makers. Many biases can, however, be introduced in such studies. Dropout and mortality are particular reasons for concern.

Most of the numerous studies of dementia incidence have been restricted to single sites. Authors have frequently attempted to assess whether rates in a given study are similar to those obtained elsewhere. However, variations between studies in the methodology employed make such comparisons unreliable. Where within-country variations in incidence have been noted, as has happened in the US, they have often been ascribed to methodological differences, but one cannot be certain whether this is the case.

Risk factors for other chronic disorders common in old age (notably cardiovascular disease and cancers) do vary in their prevalence between and within countries. In the UK, for example, the incidence of stroke is known to vary considerably across the country. A high proportion of dementia patients are thought to have a vascular component to their dementia, and it has been assumed that dementia incidence could be reduced if vascular risk were better controlled. One way to test this hypothesis is to compare sites with known variation in vascular risk to assess whether there is also variation in the incidence of dementia.

The Medical Research Council Cognitive Function and Ageing Study (MRC CFAS) is a multi-site, population-based study in the UK of individuals aged 65 years and over living in the community, including institutions. Diverse sites have been chosen, with varying exposures of potential importance in dementia. A two-phase two-wave design has been employed, with the waves two years apart. A standard set of instruments for the diagnosis of dementia is used throughout. CFAS now publishes incidence estimates from five sites, using likelihood-based methods to compare the first two waves of interviews.

Predictably, incidence rates of dementia, for both sexes, were found to rise with age, from 6.7 per 1,000 person years at age 65–69 years to 68.5 per 1,000 person years at age 85 years and above. The authors estimate that around 163,000 new cases of dementia occur in England and Wales each year. However, there was no convincing evidence of variation across sites, and the incidence rates do not reflect the variations in the prevalence of possible risk factors in these sites. We therefore cannot assume that action to reduce vascular risk will have a significant impact on dementia incidence.

Another issue addressed by the study is previous suggestions in the literature that dementia incidence rates might be lower in the oldest age groups. The limited number of respondents in these age groups in previous studies made it impossible to test this hypothesis. The CFAS, however, found no evidence of any such tailing off in incidence, which also has implications for policy and planning.

The CFAS is important because it provides the first multi-site comparison of incidence rates in ethnically homogeneous populations within a country, and within Europe, using identical methodology across sites. The methodological approach developed for the study will also be of value for researchers undertaking other studies of dementia incidence, and in other chronic disease studies involving a two-phase selection process.

Brayne C, Matthews F, Medical Research Council Cognitive Function and Ageing Study Investigators (2005) The incidence of dementia in England and Wales: Findings from the five identical sites of the MRC CFA study. DOI: 10.1371/journal.pmed.0020193