

Synopsis of Research Articles

The Complicated Art of Tracking Dengue

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Compared with malaria, dengue fever has a rather lower profile in the public mind, although to those who have had it, it leaves a great impression. The name dengue fever is derived from the Swahili words *Ki dengua pepo* (“it is a sudden overtaking by an evil spirit”), which gives an idea of the rapid onset of the disease. The dengue virus is carried by the mosquito *Aedes aegypti*, and the disease often occurs as epidemics. Although the classic illness is a fairly benign acute febrile syndrome, it may be very painful—hence the English nickname, breakbone fever. The virus can also cause a much more serious illness known as dengue hemorrhagic fever, which can progress to dengue shock syndrome. There are four main serotypes of the dengue RNA virus; dengue hemorrhagic fever is more likely to occur during dengue infection in people with preexisting active or passive (e.g., maternally acquired) immunity who are exposed to a different dengue virus serotype. In contrast to classic dengue, the hemorrhagic fever and shock syndromes are mostly diseases of children and, if untreated, have a mortality of around 50%.

Around two-fifths of the world's population are now at risk of the disease (one estimate is that 80 million people are infected each year). The number at risk will increase as population growth, urbanization, international travel, and climate change influence transmission of the disease. Understanding how all these factors interact is important in planning for disease outbreaks. However, the incidence of dengue is not easily predictable, varying with season, and also between years. For example, although dengue is most prevalent in the wet season, dengue epidemics have also been associated with drought in some countries. El Niño is the best known climatic event affecting climate between years, and some research already suggests that there is a relationship between the timing of dengue epidemics and El Niño in the Pacific Islands and in other countries.

Previous research has uncovered traveling waves of dengue in Thailand, but the cause of these has been obscure. In a paper in this month's *PLoS Medicine*



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***Aedes aegypti*, the main vector of dengue (Photo: CDC/Robert S Craig)**

Bernard Cazelles and colleagues looked at the details of the relationship between dengue incidence and El Niño in Thailand. Their results, based on complex mathematical analysis, do not provide easy answers for those who

might want to plan for dengue outbreaks, though they do go some way to helping to understand the complex interplay between the various factors. In essence, the researchers found that there was a significant association between El Niño oscillations, climate variables, and dengue hemorrhagic fever incidence with a 2- to 3-year repeat, for both Bangkok and the rest of Thailand. However this association was significant only for the years 1986–1992, and outside these years factors other than climate were probably responsible for triggering the disease outbreaks.

Cazelles B, Chavez M, McMichael AJ, Hales S (2005) Nonstationary influence of El Niño on the synchronous dengue epidemics in Thailand. DOI: 10.1371/journal.pmed.0020106

Producing Insulin from Neural Cells

DOI: 10.1371/journal.pmed.0020115

Before insulin was discovered and purified, doctors could only watch as their patients slowly died of type 1 diabetes mellitus. In January 1922, the prognosis was changed dramatically when a teenager with diabetes in a Toronto hospital became the first recorded recipient of an injection of insulin. Although this preparation was far from perfect, as Frederick Banting said in his Nobel lecture of 1925 (Nobel laureates did not have to wait so long as they do now for recognition), “There was a marked reduction in blood sugar and the urine was rendered sugar free.” Suddenly diabetes was a potentially curable disease. But insulin's very success brought trouble, as demand far exceeded the supply.

Now recombinant insulin is available in plentiful supply, but the control of type 1 diabetes remains far from perfect, and researchers have looked towards an ideal of transplanting insulin-producing cells instead. The best protocol for this, also pioneered in Canada, the Edmonton protocol, uses β -cells isolated from human cadavers and has shown some remarkable results with around 84% of patients remaining insulin-free after one

year and 89% of patients still producing insulin after three years. However, the isolation of β -cells is laborious and limited by donor availability.

The next logical step, then, is to look for renewable sources of insulin-producing cells. The emerging science of human stem cell research makes this step possible, and in a paper in this month's *PLoS Medicine* Seung Kim and colleagues from Stanford University suggest that researchers should look beyond just pancreatic precursors and embryonic stem cells to other cell-type precursors for ideas about β -cell replacement.

The rationale for the approach in this study comes from observations that although the pancreatic islet cells are the principal source of insulin in humans, in some invertebrate species, such as *Drosophila*, most circulating insulin is produced by brain neurons. Intriguingly, the gene encoding insulin is also transcribed by some vertebrate neurons—although it is not clear whether they then produce or secrete insulin protein.

Kim and colleagues took human neural progenitor cells derived from brain,



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Insulin-producing neurospheres

and exposed them to a series of signals that are known to drive pancreatic islet development. They were able to produce clusters of insulin-producing cells that were responsive to glucose in vitro. After transplantation into immunocompromised mice, circulating human insulin and C-peptide derived from the proinsulin precursor were detected when the mice were given glucose.

Of course, results in mice do not mean that such treatments would automatically work in humans, and before any such therapies become available there are many hurdles to overcome. Some of the most important include the long-term

stability and safety of the cells (although the cells remained differentiated in the mice and did not form tumors, such a risk would need to be very thoroughly investigated because of the chronic nature of diabetes), and how to scale up such a process to produce the much larger numbers of cells needed for human treatment. Nonetheless, work like this study from Kim's group might point to where the future of diabetes treatment lies.

Hori Y, Gu X, Xie X, Kim SK (2005) Differentiation of insulin-producing cells from human neural progenitor cells. DOI: 10.1371/journal.pmed.0020103

Trends in the Prescribing of Thiazides for Hypertension

DOI: 10.1371/journal.pmed.0020113

Hypertension is common in affluent societies and a major risk factor for heart disease. In Canada, hypertension is the leading primary diagnosis for patient visits to physicians' offices. Beyond recommending lifestyle changes such as losing weight, quitting smoking, and lowering salt and alcohol intake, prescription drugs are indicated in many patients. As a consequence, antihypertensive drugs are the leading category of prescription drugs in Canada, accounting for 20% of prescription drug sales.

Several classes of drugs are available for treatment, including diuretics, ACE inhibitors, and calcium channel blockers. First-line treatment with thiazide diuretics—the oldest and by far the cheapest drug class—has been shown in randomized trials to reduce serious cardiovascular morbidity and mortality with benefits at least as great as first-line treatment with other drug classes.

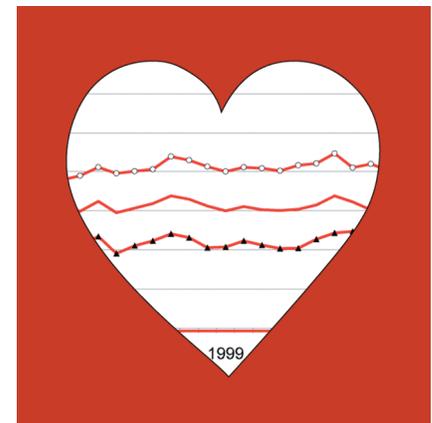
Steve Morgan and colleagues set out to examine whether prescribing practices were in accordance with this evidence. They analyzed administrative claims data from a public drug plan for seniors (residents of age 65 and older) to determine trends in first-line hypertension drug use. During the period from 1993 to 2000, over 82,000 seniors were identified as new users of hypertension drugs. Less than a third of these patients received thiazides (alone or as part of a combination regimen) as a first-line treatment.

The share of new patients receiving a thiazide increased over the study

period, but did not exceed 45% at any point. Women were more likely than men, and older patients were more likely than younger ones, to receive thiazides. Comorbidities also influenced prescribing practices: patients without concurrent diagnoses were more likely to receive thiazides. While for some comorbidities (such as previous acute myocardial infarction) evidence suggested that there were good reasons to prescribe drugs other than thiazides, no such evidence existed for many of the other conditions that nevertheless were associated with lower prescription of thiazides.

Changes in drug availability and existing evidence during the period studied make it difficult to calculate the exact extent to which thiazides were under-prescribed. However, the study shows that many patients received drugs that had previously been found to be no better at treating hypertension than much cheaper alternatives. Drug prices changed over the study period as well, but even comparing the lowest price for any of the alternatives to thiazides, \$0.34 per day, with the constant cost of less than \$ 0.01 per day for a thiazide makes it clear that a lot of money was wasted.

On a more positive note, prescription of thiazides as a first-line therapy rose over the study period—from 25% to 42% in patients without comorbidities. Most of the increase occurred shortly after a specific local education campaign. This suggests that repeated targeting of prescribing physicians—many of whom receive regular



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Prescription trends for hypertension

marketing material from pharmaceutical companies and subscribe to the general view that newer drugs are better—with current evidence-based information should be considered.

One of the most influential studies comparing antihypertensive drugs, the ALLHAT study, also supported the use of thiazides as first-line drugs. ALLHAT was published in 2003, and its results widely publicized. According to Steve Morgan, "Anecdotal evidence suggests that ALLHAT has had an influence on prescription practices, but I am not aware of a large-scale analysis yet."

Morgan S, Bassett KL, Wright JM, Yan L (2005) First-line first? Trends in thiazide prescribing for hypertensive seniors. DOI: 10.1371/journal.pmed.0020080

Monitoring HIV-1 Resistance across the Globe

DOI: 10.1371/journal.pmed.0020117

As the HIV-1 epidemic continues to grow, mutations in the virus that confer drug resistance are becoming increasingly important in the clinical management of patients worldwide. Of all the different virus subtypes (A, B, C, D, F, G, H, J, and K) and a rapidly increasing number of established and emerging recombinant viruses, it is subtype B that predominates in Western Europe, the United States, and the rest of the industrialized world. Antiretroviral drugs were developed by studying subtype B, and most data on the genetic mechanisms of HIV drug resistance are also from subtype B. However, worldwide, subtype B is in the minority (~10% of the infected population). In Africa, for example, where there is broad viral diversity, there is a greater spread of subtypes, with subtype C being the most common, representing over half of all infections. Although it seems that current drugs—developed against subtype B virus—are active against non-subtype-B virus, one critical issue is whether viruses from some subtypes or particular regions are more likely than others to develop resistance against certain drugs. Another crucial issue is to identify the mutations that confer drug resistance in non-B subtypes. Answering these questions might determine whether initial treatment strategies should be different for people with non-subtype-B viruses, and also could help decide how patients with non-subtype-B virus who fail antiretroviral therapy should be managed.

In a paper in this month's *PLoS Medicine*, Rami Kantor and colleagues from a worldwide collaboration have looked at the mutations found in 3,686 people with non-subtype-B HIV-1

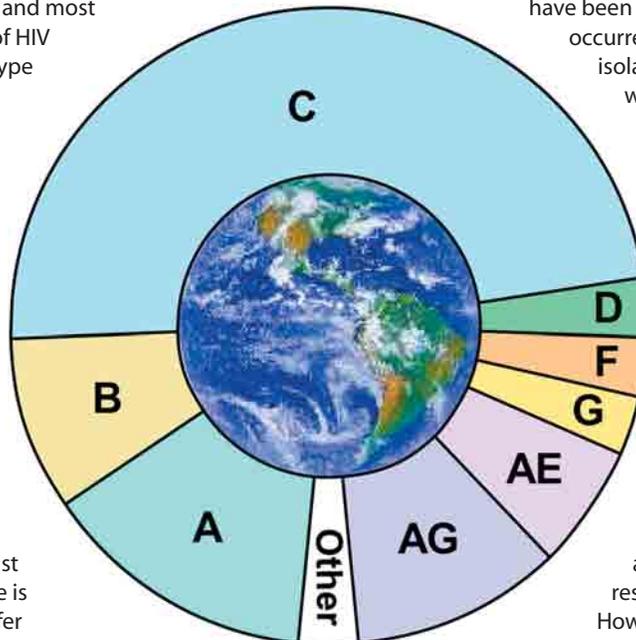
infections compared with those in 4,769 people with subtype B infections. They wanted to answer two questions: first, whether the mutations that cause drug resistance in subtype B viruses also develop in non-subtype-B viruses exposed to antiretroviral drugs, and second, whether novel mutations (i.e., not previously seen in subtype B virus) develop in non-subtype-B viruses when they fail to respond to antiretroviral drugs. What they found was

that all of the 55 drug-resistance mutations that have been known to occur in subtype B also occurred in at least one non-subtype-B isolate, and most of these mutations were also statistically associated with antiretroviral treatment in at least one non-B subtype. Conversely, of the 67 mutations associated with antiretroviral therapy in at least one non-B subtype, 61 were also associated with antiretroviral therapy in subtype B isolates.

So it appears that few novel mutations are arising in non-subtype-B viruses exposed to the current antiretroviral drugs and that the present focus on subtype B mutations for global surveillance and genotypic assessments of drug resistance is a reasonable approach. However, the authors emphasize that differences in the types and patterns of drug-resistance mutations are likely to differ between the subtypes, and that larger numbers of samples and further

analyses are needed to exclude the possibility of new and/or rare subtype-specific mutations.

Kantor R, Katzenstein DA, Efron B, Carvalho AP, Wynhoven B, et al. (2005) Impact of HIV-1 subtype and antiretroviral therapy on protease and reverse transcriptase genotype: Results of a global collaboration. DOI: 10.1371/journal.pmed.0020112



DOI: 10.1371/journal.pmed.0020117.g001

There are many subtypes of HIV-1 worldwide

Reducing Malaria Transmission in Africa

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There are 300 million cases of malaria each year worldwide, causing one million deaths. Around 90% of these deaths occur in Africa, mostly in young children. One of the greatest challenges facing Africa in the fight against malaria is drug resistance; resistance to chloroquine (CQ), the cheapest and most widely used antimalarial, is common throughout Africa, and resistance to sulfadoxine-pyrimethamine (SP), the first-developed and least expensive alternative to CQ, is also increasing in eastern and southern Africa. These trends have forced many

countries to change their treatment policies and use more expensive drugs, including drug combinations that will hopefully slow the development of resistance. One avenue of research is to identify combinations that minimize gametocyte emergence in treated cases and prevent selective transmission of parasites resistant to any of the partner drugs.

In this month's *PLoS Medicine* Colin Sutherland and colleagues tested two leading combination therapies in children with uncomplicated malaria.



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Thick blood films from patients in Gambia with malaria (Photo: Elisa Meier)

One regimen was an artemisinin-based combination consisting of artemether and lumefantrine (co-artemether, trade names CoArtem and Riamet). The other was a combination of CQ and SP—currently under consideration in several African countries, largely due to its low cost. In this randomized, controlled trial, 497 children with acute uncomplicated falciparum malaria were given either a combination of CQ and SP or six doses of co-artemether (91 received CQ/SP and 406 received co-artemether), and their blood was tested for infectivity to mosquitoes seven days after treatment. During follow up at seven, 14, and 28 days the team found that children treated with co-artemether were significantly less likely to carry gametocytes in their blood than children treated with CQ and SP—7.9% compared with 48.8%.

Altogether, the six-dose regimen of co-artemether was highly effective at reducing the prevalence and duration of gametocyte carriage. The numbers of gametocytes and the infectiousness to mosquitoes at day 7 were also reduced compared to a combination of CQ and SP, said the authors. Other studies have already shown the potential of co-artemether combination therapy to both cure malaria and reduce gametocyte carriage, acknowledged the authors. However, this study is the first to demonstrate the treatment's potential to markedly reduce the infectiousness of patients to mosquitoes, and has done so in a sub-Saharan African setting with highly seasonal transmission and where asymptomatic infections are common.

Do the results mean co-artemether should be introduced as a first-line treatment for malaria in Africa? The

authors are hesitant and suggest there might be compliance issues with the six-dose regimen. The requirement of oily food for adequate absorption might also lead to inadequate drug levels in the blood of many treated individuals.

The authors suggest that co-artemether as a first-line treatment is not likely to reduce overall transmission of *Plasmodium falciparum* within the community but rather would reduce selective transmission of resistant parasites in treated patients. Hence, co-artemether could have a public health benefit by reducing the impact of drug resistance.

Sutherland CJ, Ord R, Dunyo S, Jawara M, Drakeley CJ, et al. (2005) Reduction of malaria transmission to *Anopheles* mosquitoes with a six-dose regimen of co-artemether. DOI: 10.1371/journal.pmed.0020092

