Prediction of epileptic seizures: are nonlinear methods relevant?

To the editor—Epilepsy is one of the most common serious neurological disorders, affecting 1% of the population at some time. Reliable and robust detection of seizure precursors would improve the quality of life of many epilepsy sufferers. It is likely that the processes underlying the electroencephalogram (EEG) signal are nonlinear[1,2], but there is little, if any, concrete evidence that such signals reflect deterministic chaos. Regardless of fundamental dynamics, the relevant operational question is whether or not the information reflected in a proposed test statistic justifies its use (given its complexity). Can a complicated, novel and potentially nonlinear method systematically out-perform traditional 'linear' methods such as analysis of variance, or provide independent and complementary precursor information?

One statistic[3] capable of detecting nonlinear correlations that are invisible to variance is the correlation density, $C(r_0)$: the fraction of pairs of points whose separation is less than a specific distance r_0. The correlation density is a geometrical measure of the clustering of points. $C(r_0)$ may also reflect changes in variance; for example, increasing the variance of a given distribution will decrease $C(r_0)$. In a widely cited paper, Martinerie *et al.*[4] calculated $C(r_0)$ for EEG recordings and reported that $C(r_0)$ identified precursors to epileptic seizures in 10 of the 11 patients considered. A comparison between $C(r_0)$ and variance for a number of these recordings (Fig. 1) shows that, as expected, changes in $C(r_0)$ are echoed by changes in variance. This suggests that there is little justification for using $C(r_0)$ instead of variance as a statistic for predicting the epileptic seizures in this database.

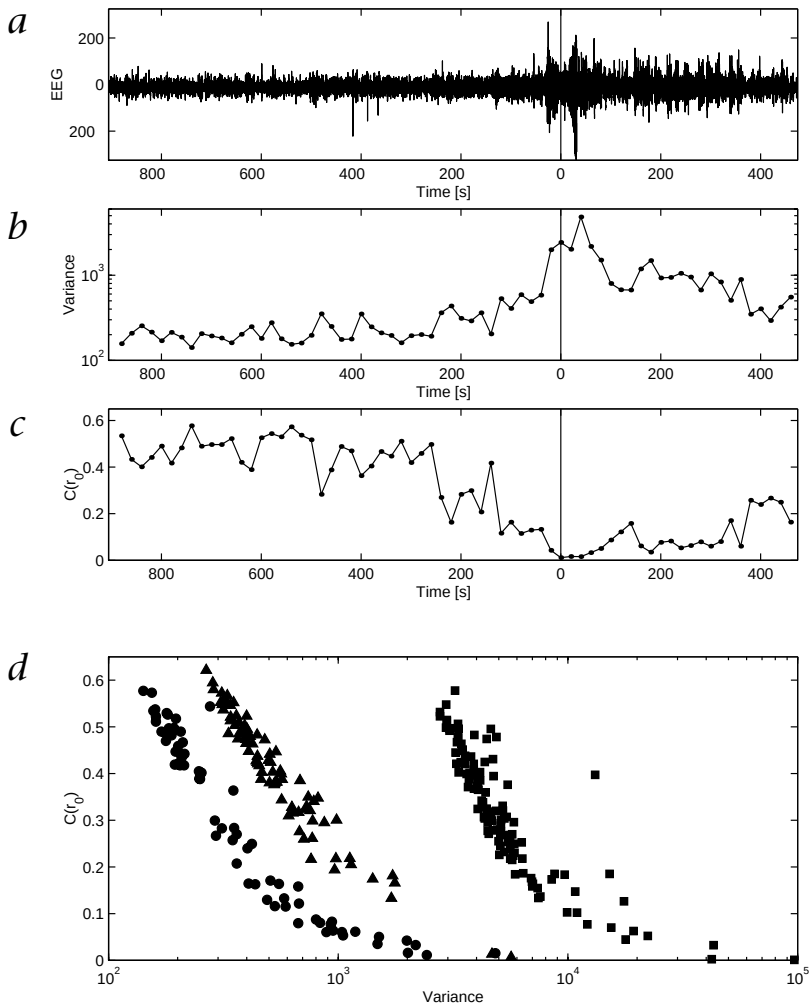

Fig. 1 Results based upon the database investigated by Martinerie *et al.*[4]. ***a***, EEG from a single channel of recording 8/1 (subject/seizure). ***b***, Moving variance. ***c***, Correlation density $C(r_0)$ using 20-s windows. Note the change in behavior prior to the seizure onset in both $C(r_0)$ and variance. Note also the logarithmic scale for the variance. ***d***, Comparison of $C(r_0)$ with variance for recordings 1/1 (■), 8/1 (●) and 11/1 (▲). The clear relationship between $C(r_0)$ and variance indicates that in these cases, there is little information from one that is not available from the other.

Surrogate data tests[5] provide a measure of significance against a specific null hypothesis; the choice of a relevant null hypothesis is crucial[6,7] if the results of the test are to be applied in practice. The aim here is not to detect nonlinearity, but to establish the test's skill in detecting precursors. Martinerie *et al.* "constructed multivariate surrogate data from each raw data set in such a way that the linear correlation within each component time series and the cross-correlation between them is preserved." Using the entire duration of a recording in this way implies the null hypothesis that the variance is constant. This does not seem likely, as can be seen in one of the EEG recordings with onset of seizure at time zero (Fig. 1*a*). The increase in variance before the seizure is striking. The fact that the variance is constant in the surrogates but not in the real data implies that the value of $C(r_0)$ for the original recording will differ from these surrogates simply because the variance changes. If a group of such surrogates (data not shown) is contrasted with the real signal on which they were based, the real signal can easily be identified by eye. Thus it is not surprising that the values of $C(r_0)$ calculated for the real recording (Fig. 2 of ref. 4) are wildly different from those calculated for the surrogates. The result is 'statistically significant', but the null hypothesis is physiologically irrelevant; changes in variance (heteroscedasticity) can be as easily detected by monitoring the variance. We stress the need

for tests of relevant null hypotheses[6,7] and their immediate relation to the detection method in the diagnosis of medical disorders. Ultimately, the operational use of proposed complicated statistics can be justified only by showing that they out-perform well-understood traditional statistics (such as variance) or provide complementary information. The fact that the signal itself may be demonstrably nonlinear is simply not the relevant question when event detection is the aim.

To establish the efficacy of any new detection approach to medical diagnosis, we argue first for surrogate data tests against a null hypothesis relevant to some simple traditional statistic, and second for quantification of the false alarm rate. In the present case, the first point could be addressed using surrogates that preserve the temporal variation in the variance; the second point would require an experimental design including long records of seizure-free data.

Competing interests statement
The authors declare that they have no competing financial interests.

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Martinerie et al. reply—Until 1998, neuroscientists thought that epileptic seizures began abruptly, just a few seconds before clinical onset. It was during that year that two independent studies[4,8] showed that the non-linear time series analysis of EEG data could reveal dynamical changes several minutes before seizure onset. The usefulness of non-linear measures for the detection of pre-ictal changes has since been confirmed[9]. This new approach has opened a new field of seizure anticipation and defined a framework for better understanding of seizure generation mechanisms.

McSharry *et al*. have re-analyzed our 1998 database and have shown that the non-linear index is sensitive to amplitude variance fluctuation. We have been aware of this limitation for some time now. We developed, in 1999, a new method[10] that did not involve the reconstruction of the dynamics from the amplitude of the signal, presenting a number of practical advantages over our previous method. The new method measures similarity to quantify the extent to which the EEG dynamics, reconstructed from the phase information, differ between periods taken at distant moments in time. The phase is defined as the time between two successive zero-crossing intervals. This relative measure reveals the spatial distribution of pre-ictal dynamic changes (both linear and non-linear) that involve the epileptogenic area but do not seem to be confined to the restricted ictal onset region. Furthermore, it is very robust against noise and artifacts, and fast enough to be carried out in real time.

The surrogate data that we had selected for the 1998 study to test the presence of deterministic structure in the time series[4] had been built for each block of data (20 s; this may not have been not clear in the paper) and were designed to reject a null hypothesis of a non-linear transformation of linearly filtered noise. Thus, the variances of the raw data and surrogate data were the same. We found a statistical difference between the values of $C(r_0)$ calculated from the raw data and those calculated from the surrogate data, which led us to reject this null hypothesis. We know that one should be extremely careful with the use of surrogate procedures (which can be very sensitive to the presence of spikes in the data and detect spurious non-linearity[11], for example). It is advisable to obtain consistent results with more than one type of surrogate, to get an indication of non-linear deterministic structure. New strategies and algorithms are now available[12].

In conclusion, our recent results[13,14] using the similarity method support the idea that pre-ictal dynamic changes (either linear, non-linear or both) have a higher probability of occurring before epileptic seizures. As previously reported[15], McSharry *et al*. suggest that some linear methods can detect pre-ictal changes in a manner similar to non-linear methods. Both analyses probably constitute different ways of viewing the same thing and some combination of them will be a good method for reliable seizure anticipation. Real progress may require collaboration between research groups, which has already begun in an international program (special interest group session on engineering and epilepsy, 56th Annual Meeting of the American Epilepsy Society, Seattle, Washington, December 6–10, 2002; and the First International Conference on Seizure Anticipation, Bonn, Germany, April 24–27, 2002).

Competing interests statement
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Humoral immunity and atherosclerosis

To the editor—The re-evaluation of atherosclerosis as a chronic inflammatory disease represents a major change in our understanding of the pathogenesis of this disease and has substantial implications in therapy and prevention. The publication of a special section on "Advances in Atherosclerosis" in the November 2002 issue of *Nature Medicine* was rather timely. However, the otherwise excellent review, "Innate and acquired immunity in atherogenesis,"[1] was strongly biased in favor of the postulate that lymphocyte-mediated inflammation is primarily responsible for plaque development and that, in contrast, the humoral responses to modified lipoproteins may be protective. However, there is a wide body of evidence contradicting this position that needs to be evaluated in a more balanced and objective perspective.

The proposed protective role for low-density lipoprotein (LDL) antibodies is based on experimental studies summarized by Binder *et al.*[1] For example, Apo-E–deficient and LDL receptor–deficient mice immunized with homologous malondialdehyde-modified LDL (MDA-LDL) showed a reduction in the development of atheromatous lesions[2,3]. Similar observations were reported in hypercholesterolemic rabbits immunized with oxidized autologous LDL[4]. More recently, it was reported that a human-derived monoclonal antibody against oxidized LDL (oxLDL) inhibited the uptake of oxLDL by macrophages[5]. However, these results need to be evaluated in a more critical way, taking into consideration significant contradictions in the immunization data. In Ameli's study[4], for example, antibodies against oxLDL developed spontaneously in non-immunized animals as well as in animals immunized with native LDL. The greatest 'protective' effect of immunization was observed in animals immunized with native LDL. The same protective effect of immunization with native LDL was observed in LDL receptor–deficient mice, where the reduction in atherosclerosis development was seen in the absence of antibodies to modified LDL[6]. Finally, the protective effect of human-derived autoantibodies against oxidized LDL reported by Shaw *et al.*[5] was artifactual, resulting from the use of incomplete antibody molecules (Fab fragments) that were unable to activate complement or interact with Fc-γ receptors.

There is rather substantial evidence indicating that antibodies against modified LDL and, particularly, the antigen-antibody complexes they form, have a pathogenic role in human atherosclerosis. Human antibodies against oxLDL and advanced glycation endproduct (AGE)-LDL have been isolated from human sera[7,8]. In contrast with mouse and rabbit oxLDL-specific antibodies[1], human oxLDL-specific antibodies are predominantly IgG, of the pro-inflammatory and Th2-dependent IgG1 and IgG3 subclasses[7]. Similar class and subclass distributions were found among purified human antibodies against AGE-LDL[8].

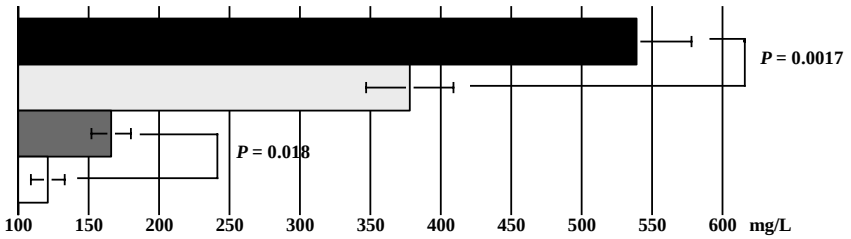

Fig. 1 The levels of cholesterol and ApoB in isolated immune complexes (IC) are related to the development of coronary artery disease. Cholesterol and ApoB were measured as surrogates for LDL-IC in immune complexes isolated from two matched cohorts of diabetic patients[9]. The cohort designated as 'cases' corresponds to 49 patients with type-1 diabetes mellitus that developed coronary artery disease during an eight-year period after collection of the assayed serum samples. The cohort designated as 'controls' corresponds to 49 patients with type-1 diabetes mellitus who remained free of coronary artery disease during an identical follow-up period. The values were compared by unpaired 2-way Student's *t*-test analysis. ■, IC cholesterol (cases); ■, IC cholesterol (controls); ■, IC ApoB (cases); □, ApoB (controls).

Although there is conflicting data concerning the pathogenicity of circulating modified LDL-specific antibodies[9], there is strong data supporting the involvement of LDL-immune complexes (LDL-IC) in the pathogenesis of atherosclerosis. Ylä-Herttuala *et al.* purified oxLDL and the corresponding antibodies (IgG and IgM) from atheromatous lesions of humans and Watanabe hyperlipidemic rabbits, thus demonstrating that the ingredients necessary for the formation of LDL-IC are present in the damaged arterial wall[10,11]. The atherogenicity of LDL-IC has been proven by experimental studies using isolated circulating LDL-IC or LDL-IC prepared *in vitro*. Isolated immune complexes containing antibodies against LDL and oxLDL can elicit cholesterol ester accumulation in macrophages, a property shared by model immune complexes prepared with oxLDL and rabbit antibodies against LDL, and by immune complexes prepared with human oxLDL and purified human oxLDL-specific antibodies[12]. The phagocytes ingesting LDL-IC after Fc-γ receptor engagement become activated and release TNF-α, IL-1, oxygen-active radicals and matrix metalloproteinase-1 (ref. 12).

Epidemiological support for the pathogenicity of LDL-IC was produced by a prospective study of 98 diabetic subjects recruited as part of the Diabetes Control and Complications Trial and the Epidemiology of Diabetes Interventions and Complications study. The data showed that high concentrations of LDL-IC correlated with the development of coronary artery disease over a period of eight years[9] (Fig. 1). This observation is particularly significant because it is the only prospective study that has investigated the correlation between the levels of circulating oxLDL and the development of coronary heart disease; this type of study is considerably more powerful than previously reported cross-sectional studies.

In conclusion, the role of the immune system in the pathogenesis of atherosclerosis is complex and cannot be oversimplified in terms of a dichotomic interaction in which Th1-mediated processes are deleterious and Th2-mediated responses are protective. Both types of response have pro-inflammatory potential, and whereas the

data obtained in experimental animals suggests that the inflammatory process is mainly dependent on the activation of Th1 responses, the data collected in human studies suggests that humoral immunity has an equally significant pathogenic role.

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Binder et al. reply—We are surprised by the letter by Virella *et al*. We did not paint the simple picture that atherogenesis was due to lymphocyte-mediated inflammation and that all humoral responses to modified lipoproteins were protective. Our review focused mainly on the results from animal studies that assessed the role of certain immune mediators in general, rather than antigen-specific ones. There are few experimental studies specifically studying the role of antibodies in atherogenesis *in vivo*, be they antibodies to modified lipoproteins or to other antigens. Therefore, and because of space restrictions, we only briefly discussed the complex roles that humoral immunity might have. In our opinion, however, there are likely to be both protective and adverse consequences of humoral immunity as well, not simply negative ones as suggested by Virella *et al*. The main message of our brief review was that immunological responses do influence atherogenesis in experimental animal models, and that all immunological responses are complex, potentially exerting both protective and adverse consequences.

With regard to antigen-specific responses, it was our laboratory that first showed the protective effect of immunization with oxLDL, but we have not indicated that the protective effect is necessarily due to humoral immunity, as implied by Virella *et al*. We invite them to review our papers, as we even stated in the title of one manuscript that the protective effect of immunization occurred "by mechanisms other than induction of high titers of antibodies to oxidative epitopes"[6]. Virella *et al*. did not mention the study by Zhou *et al*.[13], which showed an inverse correlation between lesion size and T-cell-dependent antibody titers in MDA-LDL immunized mice.

The idea that plasma immune complexes involving modified LDL are atherogenic is an old one, as discussed by Beaumont[14], Klimov[15] and Orekhov[16]. We do not disagree with the potential for such immune complexes to cause macrophage activation and increased cholesterol accumulation *in vitro*, as previously shown[14–17]. However, the presence of such immune complexes in plasma does not necessarily indicate atherogenicity *in vivo*. For example, in the interesting epidemiological observation in diabetics cited in the letter by Virella *et al*., the epitopes on LDL to which the antibodies bind were not studied. Presumably the autoantibodies were against modified, and not native, LDL. We showed previously that there was enhanced nonenzymatic glycation of LDL in diabetics[18], and that autoantibodies directed to the glucose-lysine adducts on the glycated LDL were present in diabetic subjects and led to immune-mediated clearance[19]. In an experimental rabbit model, the presence of such autoantibodies effected the rapid removal of such glycated LDL to macrophage-rich organs such as the liver, spleen and bone marrow, and away from the artery wall[20]. Thus, while such immune complexes may be a marker for disease, and may even cause increased cholesterol accumulation in macrophages *in vitro*, they may not necessarily have a net atherogenic effect *in vivo*.

The atherogenicity of antibodies may well be context- and antigen-dependent. For example, several investigators reported that the presence of IgM against oxLDL was inversely associated with risk[21–23]. Major *et al*.[24] have just shown that B-cell deficiency in *Ldlr*$^{-/-}$ mice leads to enhanced atherogenesis. Caligiuri *et al*.[25] have shown that splenectomy, which decreased antibody production to oxLDL, increased atherogenesis in *Apoe*$^{-/-}$ mice, and that this adverse effect could be completely rescued by B-cell transfer, which restored antibody production. Since B cells are not normally found in lesions, these studies suggest a protective role of humoral immunity under the conditions of these experiments. Finally, using a clearly defined specific example of a natural IgM antibody against oxLDL, EO6, we pointed out in our review the theoretical possibility that such an antibody could be protective, as it inhibited the uptake of oxLDL by macrophages. This antibody is also known to provide optimal protection to mice against lethal infection with *Streptococcus pneumoniae*, which, like oxLDL, displays the phosphorylcholine (PC) epitope. In the letter by Virella *et al*., the reference to the paper by Shaw *et al*.[5] is inappropriate, as we did not cite this paper in our review. The purpose of that paper was to show that the epitope of the first cloned human autoantibody to oxLDL, a Fab prepared from a human Ig phage-display library, was a ligand mediating the uptake of oxLDL.

We stress again that we are not advocating a generalized protective role of humoral immunity in atherogenesis. The possibility that antibodies, not only to modified LDL but also to heat shock proteins or infectious pathogens, could provoke pro-inflammatory and pro-atherogenic responses is reasonable, and was specifically mentioned in our review. However, these pro-atherogenic possibilities, which were inferred from *in vitro* data, remain to be shown *in vivo*, as do the potentially protective roles cited above.

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How to submit microarray data

Nature Medicine has implemented a new policy regarding microarray experiments as of 1 December 2002. As discussed in a recent editorial in *Nature* (**419**, 323; 2002), *Nature Medicine* will now require authors to submit microarray data in accordance with the Minimal Information About a Microarray Experiment guidelines issued by the Microarray Gene Expression Data society. The guidelines include a checklist of relevant information that should be included with every new microarray submission, and can be found online at http://www.mged.org/Workgroups/MIAME/miame_checklist.html. The supplementary information must be supplied with the manuscript on five compact discs, at the time of submission, in a format compatible with commonly available software packages. We will also require that data central to the paper's conclusions be deposited in a public database for microarray data and accession numbers provided, where available, at or before acceptance for publication. By adopting this policy, we hope that the explicit description of experimental design and methods will facilitate the review and replication of microarray results.