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Against

Differences between groups or changes between conditions in normalized tissue radioactivity following administration of a cerebral blood flow (CBF) tracer cannot be interpreted as true differences or changes in regional cerebral blood flow (rCBF) without increased risk of false positive and false negative findings. The risk arises from two factors: (1) changes in rCBF are not equivalent to changes in tissue activity unless there exists a strictly linear relationship between the two; and (2) changes in normalized rCBF do not translate into changes in rCBF unless CBF in the reference region is unchanged. Some of the pitfalls of using normalized activity are discussed below and problems inherent in this approach are illustrated with simulated data and examples from the literature.

Why apply normalization?

There are unquestionably situations, particularly in some clinical settings, when quantification of blood flow is unnecessary, e.g., an image of tissue radioactivity itself may be sufficient to identify the presence and extent of a pronounced rCBF deficit. Studies investigating more subtle changes, e.g., differences among different populations or changes in blood flow under differing experimental conditions, on the other hand, require some form of quantification in order to compare different subjects and conduct statistical inference testing; the present discussion pertains to these studies.

Tissue tracer activities cannot be directly compared since they depend not only on rCBF, but also on the level of tracer in the blood which supplies the brain. The arterial input function varies from study to study even in the same individual, e.g., with changes in cardiac output or its distribution among the organs of the body. Thus it is possible to observe changes in tissue activity in the absence of changes in regional or global CBF (gCBF). Comparisons based on quantitatively determined absolute values of rCBF (in ml (100 g)$^{-1}$ min^{-1}, for example) avoid this problem. Regional tissue activities normalized to activity in a reference region (such as whole brain or cerebellum) are also used; in these analyses CBF is assumed to be constant in the reference region. Initially, the latter type of testing was applied in PET activation studies with the whole brain used as the reference region and the assumption that gCBF was unchanged between the resting or control and the test states. The assumption was that changes in rCBF are regionally selective and suffi-

ciently small in either spatial extent or in magnitude so as not to affect the average CBF of the brain as a whole. The testing of activity ratios has since been extended to comparisons of groups in which there is no a priori reason to expect constancy of gCBF, and this leads to difficulty in interpreting observed changes. If gCBF is decreased by 20% in a given condition but rCBF is decreased by only 10%, the ratio of regional to global tissue activity actually increases. How can this be distinguished from a true increase in rCBF unless gCBF itself is measured?

Arguments in favor of normalization generally fall into two categories: (1) difficulties in obtaining the arterial input function required for absolute rCBF quantification; and (2) reduction in variance that results from normalization. The first argument is a practical one in which the benefits of a quantitative study must be weighed against the cost to the patient of an invasive arterial blood sampling procedure; for certain populations arterial blood sampling is not a feasible option. Several techniques have been proposed to determine noninvasively the time course of arterial blood activity, e.g., external tomographic monitoring around a patient's neck or wrist [1], use of standard input functional shapes with a single calibrating blood sample [2], or estimation of blood activity from time-activity curves in multiple brain regions [3, 4]. Improvements in these techniques may render invasive arterial blood sampling procedures less of a factor in the future. The second often-cited reason for normalization is the reduction in variance that results from positive correlations between regional and global CBF, and hence also between regional and global tissue activities. Indeed, if gCBF is not changed and if rCBF and gCBF are sufficiently positively correlated, then use of normalized counts does lead to an increase in power to detect regional changes. The accuracy of statistical tests to identify changes in rCBF, however, depends critically on whether mean CBF in the reference region is the same in the groups under comparison. Sensitivity of the tests also depends on the relationship between rCBF and normalized tissue activity.

Relationship between rCBF and tissue activity

The relationship between rCBF and tissue activity is well known to be nonlinear; higher distribution volumes (V_D, the ratio of influx to efflux rate constants) and shorter scanning times result in a more linear relationship, whereas smaller V_D and longer scanning times result in less linearity. Fractional changes in tissue activity (and normalized tissue activity) are smaller in magnitude than fractional changes in rCBF; this limits sensitivity to detect changes, particularly at high blood flow levels, regardless of whether rCBF or normalized tissue activities are compared. The degree of scale compression, however, varies with the regional blood flow in a manner that is different between individuals, and between studies in a single individual due to changes in the shape of the arterial input function; this impacts only on tests of normalized tissue activity. The variable nature of the nonlinear relationship between the variate of interest (rCBF) and the variate used in testing (normalized tissue activity) creates distortions in the results of inference testing.

Tests of normalized tissue activity when gCBF is constant

To use tests of normalized activity to investigate changes in rCBF, one assumes that gCBF (or CBF in the reference region) is the same in the groups under comparison. Ideally, one would measure gCBF and utilize a test that is sufficiently powerful for the level of measurement noise and underlying biological variability to validate the assumption. Small differences in gCBF are difficult to detect, however, and to detect them with high probability may require fairly large sample sizes. Even small changes in gCBF can have a strong effect on detection of regional changes.

When gCBF is unchanged, what is the probability of detecting changes in rCBF by testing normalized tissue activity? The exact probability depends on a number of factors, including rCBF and gCBF, the relationship between rCBF and normalized activity, number of subjects, variability of rCBF and gCBF, and measurement noise. In general, when quantitatively determined rCBF measurements are compared, the power of the test to detect increases in rCBF is higher the greater the increases, as one would expect (Fig. 1A). When rCBF and gCBF are uncorrelated and normalized tissue activities are tested, the power of the test is always lower than the power of the test that compares quantitatively determined rCBF. Higher positive correlations between rCBF and gCBF increase the power of the test of normalized activities until eventually the power exceeds that of the test of quantitatively determined rCBF. In all cases type II error, the probability of falsely detecting an increase in rCBF, remains within the specified level of the test. Analogous results are found when testing for decreases in rCBF. When gCBF is constant, therefore, false positives are adequately controlled, but when correlations between rCBF and gCBF are low, the generally low power of the tests of normalized activity implies that more false negatives are to be expected than when rCBF itself is tested.

Tests of normalized tissue activity when gCBF is changed

If no quantitative measurements of gCBF are made, or statistical tests employed are not sufficiently powerful to detect actual changes, there is the obvious possibility that the mean gCBF in the two groups is different. When gCBF increases, the power of the test of normalized activities to detect increases in rCBF is greatly reduced (Fig. 1B); increases in rCBF are virtually undetectable

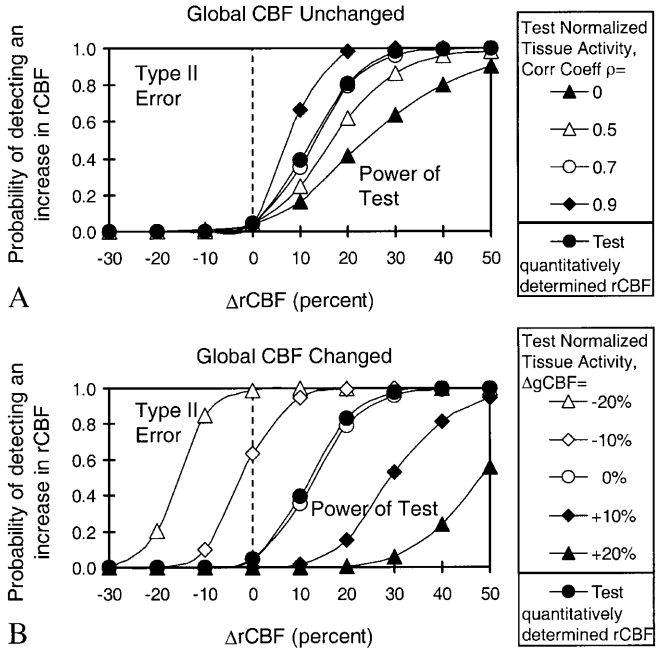

Fig 1. A Probability of detecting an increase in rCBF between two groups when gCBF is unchanged. Both quantitatively determined rCBF and normalized tissue activities were tested. On the abscissa is ΔrCBF, the true change in mean rCBF from group 1 to group 2. On the ordinate is the simulated probability of detecting an increase in rCBF. When rCBF itself is tested (*closed circles*), the power of the test to correctly detect increases in rCBF improves as the magnitude of the increase in rCBF rises. When normalized tissue activity is tested and there is no correlation between rCBF and gCBF (ρ=0, *closed triangles*), the power of the test to detect increases in rCBF is less than the power of the test of quantitatively determined rCBF. The power of tests of normalized tissue activity increases with increasing positive correlation between regional and global CBF (ρ=0.5, *open triangles*; ρ=0.7, *open circles*; ρ=0.9, *diamonds*). When rCBF and gCBF are sufficiently positively correlated, the power of the test of normalized activities exceeds that of quantitatively determined rCBF. The false positive rate, i.e., detection increases in rCBF when there is either no change or a decrease in rCBF (type II error) remains below the specified level of the test ($P<0.05$). The generally lower power of the tests of normalized activity when correlations between rCBF and gCBF are low implies that more false negatives are to be expected than when rCBF itself is tested. Data represent an $H_2{}^{15}O$ study in which ten subjects in each group were scanned for 2 min, mean gCBF and rCBF in group 1 were 50 and 80 ml $(100\ g)^{-1}\ min^{-1}$, respectively, and the coefficient of variation in CBF was 15%. An unpaired one-tailed Student's *t* test was used. **B** Probability of detecting an increase in rCBF between two groups when gCBF is changed. Both quantitatively determined rCBF and normalized tissue activities were tested. ΔrCBF is on the abscissa and the probability of detecting an increase in rCBF is on the ordinate. Regional and global CBF were assumed to be positively correlated (ρ=0.7). The true change in gCBF ranged from –20% to +20%. When gCBF increased (ΔgCBF =+10%, *closed diamonds*; ΔgCBF =+20%, *closed triangles*), the power of the test of normalized activities to detect true increases in rCBF was less than the power of the test of quantitatively determined rCBF (*closed circles*). Hence, more false negatives occur. Type II error remains below the specified level of the test ($P<0.05$). When gCBF decreased (ΔgCBF =–20%, *open triangles*; ΔgCBF= –10%, *open diamonds*), the power to detect true increases in rCBF is very high, approaching 100%. The type II error, however, also increases substantially; decreases in rCBF have a very high probability of being falsely detected as increases. Simulation data are the same as in **A**

when they are in proportion to increases in gCBF. When a decrease in gCBF occurs which is greater in magnitude than a decrease in rCBF, tests of normalized tissue activity have a high probability of detecting an *increase* in rCBF; the probability of false negatives (type II error) becomes uncontrollably high. Analogous false detection of decreases in rCBF also occur when rCBF increases less than gCBF. The outcome of the test, therefore, depends on the change in rCBF relative to that in gCBF. Decreases in rCBF can be interpreted as increases and vice versa, and both can fail detection completely.

Examples

The foregoing discussion has illustrated the possibility of encountering additional false positive and false negative results from testing normalized tissue activities rather than rCBF itself. To what extent is this actually happening in practice? There are few examples where analyses are available of both quantitatively determined rCBF and normalized tissue activity.

In a number of studies gCBF has been reported to decrease in normal aging. In a recent $H_2{}^{15}O$ PET study, for example, mean gray matter rCBF decreased by ~15% between age 25 and age 65 [5]; changes in rCBF ranged from –5% to –24% in the various gray matter regions examined, and were –13% in the cerebellum. Had the normalized tissue activity ratio been analyzed in these same subjects, one would expect to find an increase in the ratio with age in those structures in which the blood flow declines by less than in the reference region. These structures would appear, therefore, to have increased blood flow. When regional uptake of ^{99m}Tc-HMPAO or ^{99m}Tc-ECD relative to whole brain was measured with SPET, the occipital region was reported to have a "higher rCBF in the aged" [6] and a positive correlation with age [7]. Is this due to declining gCBF? In contrast with the $H_2{}^{15}O$ PET study in which quantitatively determined rCBF showed decreases with age in all structures examined, many of the brain regions examined in the SPET studies showed no change in rCBF between young and old [6] or no correlation with age [7]. Could the ratio method be overlooking important changes?

More substantial decreases in gCBF, on the order of 25%–40%, have been reported in slow wave sleep compared with the awake resting state (reviewed in [8]). Decreases in normalized tissue activity can therefore be safely interpreted as reflecting true decreases in rCBF. Regions in which activity ratios increase or do not

change, however, may also represent areas of significant decrease in rCBF that could be missed. Kajimura et al. reported marked decreases in rCBF in neocortical regions during deep non-REM sleep that were not detected by relative rCBF analysis [9].

Conclusions

As changes in rCBF are not equivalent to changes in tissue activity, and changes in normalized rCBF are not equivalent to changes in rCBF (unless CBF in the reference region is unchanged), it is important to identify precisely what is measured in each study. In the literature, measurements of quantitatively determined rCBF and normalized tissue activities have both been labeled as measurements of rCBF; this is certainly a source of confusion when study results are compared. Caution is called for in interpreting statistically significant changes in ratios of tissue activity, particularly when there is no evidence that gCBF is unchanged. When gCBF is known to decrease (increase) we can find structures in which rCBF decreases (increases) to a greater degree, but other significant regional changes may be missed. Ultimately, changes in normalized tissue activities, or lack thereof, depend on the relative changes in CBF in the region of interest and reference region. To unequivocally identify changes that are due strictly to changes in CBF in the region of interest requires quantitative determination of rCBF.

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Can normalized tissue activities be used instead of absolute blood flow measurements in the brain?

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This article was originally published under the incorrect title "Can ROI methodology/normalised tissue activities be used instead of absolute blood flow measurements in the brain?" The position "Against" normalization was not a position against the use of ROI methodology; on the contrary, the author strongly advocates region of interest analyses for cerebral blood flow data.

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