Patients with Carotid Intraplaque Hemorrhage Have Higher Incidence of Cerebral Microbleeds

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Abstract

Background

Intraplaque hemorrhage (IPH) is a strong marker of atherosclerotic plaque vulnerability. Cerebral microbleeds (CMBs) represent hemorrhage-prone small vessel disease, and are a common finding on MRI in patients with cerebrovascular disease. However, any connection between carotid artery IPH and CMBs remains scantly investigated. This study aimed to investigate whether imaging or histologic evidence of IPH is related to CMBs.

Methods

This retrospective study enrolled 101 consecutive patients undergoing CEA with symptomatic (including ischemic stroke, TIA, and amaurosis fugax) or asymptomatic ipsilateral carotid artery disease. Carotid plaque specimens were collected at CEA from all the patients and stained with Movat Pentachrome to identify the presence and the extent (%) of IPH. Neck CTA was obtained to measure the degree of carotid stenosis. Brain MRI was pre-surgically performed and CMBs were studied using T2*-weighted gradient-recalled echo (GRE) or susceptibility-weighted imaging (SWI) sequence. The CMBs were counted and localized. Clinical and biochemical data, comorbidities, and medications were recorded. The association between IPH and CMBs was examined adjusted for other risk factors.

Results

IPH was in 57 (56.4%) patients. CMBs were more observed in patients with carotid IPH compared to those without IPH [19 (33.3%) vs 5 (11.4%); p=0.010]. Logistic regression analysis demonstrated an association between the extent of IPH in the carotid atheroma and the presence of CMBs [OR 1.051 (95% CI 1.012-1.090); p=0.009]. Moreover, the carotid IPH extent was associated with the number of CMBs (p=0.004). In patients with CMBs, the median degree of ipsilateral carotid stenosis was 40 % (35-65%) and it was 70 % (50-80%) in those without CMBs, with a significant difference between the two groups (p=0.049).

Conclusions

CMBs are associated with both the presence and amount of carotid IPH, and could represent an indirect marker of vulnerable atherosclerotic disease in the carotid system.

Introduction

Intraplaque hemorrhage (IPH) in carotid atherosclerotic artery is an accumulation of blood components within the atheromatous plaque (1). It is defined as an area of the carotid plaque tissue with blood elements where erythrocytes and fibrin were found extravascular, adjacent to neovessels (2) within the necrotic core, or areas of atherosclerotic plaque (3). Histologically, Movat Pentachrome staining has been used to identify IPH in atherosclerotic plaque samples (4). IPH is considered a strong marker and relevant indicator of histologically defined plaque vulnerability leading to cerebrovascular ischemic events (5-8).

Cerebral microbleeds (CMBs) are small areas of hemosiderin deposition and represent hemorrhage-prone small vessel disease and they are increasingly recognized on MRI in patients with cerebrovascular disease. Histopathological correlation of MRI-observed CMBs has identified the majority of CMBs as recent or old microhemorrhages (9). CMBs can be detected in healthy individuals, in the elderly population, and in patients with cognitive impairment. They are also a common finding in patients with ischemic stroke (10-12) and intracerebral hemorrhage(13). However, little is known about the development of CMBs in patients with ischemic stroke (14). In addition, CMBs are considered markers of cerebrovascular risk, which may vary based on their location and underlying conditions, and strong predictors for future risk of stroke (10, 15). Although vascular risk factors have been related to CMBs (16), the natural history of these MRI lesions, their progression, and their potential role as diagnostic and prognostic markers have not been elucidated (17).

The characteristics of carotid lesions have been considered relevant in the analysis of the relationship between carotid artery atherosclerosis and the presence of CMBs (18). CMBs have been associated with the presence of carotid artery fatty plaque (18) and with an increased volume of the fatty component of the plaque (19), suggesting that the composition of the carotid plaque may be considered as a marker of the presence of CMBs. More recently, a correlation between CMBs and vulnerable carotid plaque defined on imaging has been identified (20). Others have related advanced measures of carotid atherosclerosis, such as calcification assessed using computed tomography, to presence of CMBs, indicating an association between carotid calcification and CMBs (21). Moreover, IPH has been associated with an elevated total burden of cerebral small vessel disease in patients with carotid stenosis (22).

We have previously reported the prevalence of IPH in patients with nonobstructive carotid disease (23), and in this study we further explore the potential mechanisms of cerebrovascular ischemic events in patients with carotid IPH. Although microbleeds may be not exclusively of a single vascular district, there are no data about the relationship between carotid and cerebral

microbleeds and about the association between carotid stenosis and CMBs. Therefore, the question of whether CMBs are an expression of diffuse vasculature disease or the consequence of other factors, such as carotid plaque composition, is still debated (24) and the potential association between carotid IPH and CMBs has not been investigated yet.

In this study, we hypothesized that patients with histologically defined carotid IPH would have a higher risk of CMBs. Thus, in patients who underwent carotid endarterectomy (CEA), we aimed to investigate whether the presence and the extent of carotid IPH are related to the existence CMBs. We tested our hypothesis by analyzing CMBs on brain MRI and its association with the presence and characteristics of histologically defined IPH in carotid artery plaques from patients undergoing CEA.

Methods

Study design and patient population

The institutional review board approval for this study was obtained. All the patients provided written informed consent. The study consisted of consecutive patients who underwent CEA at our institution from September 2002 to July 2021. We included all the patients who met the following criteria: 1) Participants imaged with T2*GRE or SWI sequences on pre-surgical brain MRI; 2) carotid plaque specimen collected at CEA. We excluded incomplete brain MRI, suboptimal image quality or MR images with artefacts, and the carotid plaques specimens not representative of the whole plaque. Baseline clinical characteristics and radiologic findings were retrospectively abstracted from the medical records and analyzed. The clinical data included gender, age, cardiovascular risk factors, coexisting comorbidities, and laboratory data. The use of medications at CEA and at the time of the cerebrovascular ischemic event leading to surgery were reported. Antiplatelet treatment was defined as use of aspirin and/or clopidogrel. Symptomatic were defined the patients who experienced ipsilateral cerebrovascular ischemic event such as stroke, transient ischemic attack (TIA) and/or amaurosis fugax, diagnosed after a neurological, neurosurgical and radiological evaluation based on previously described criteria (25-28); negative comprehensive stroke workup showed no other thromboembolic source and we excluded individuals with nonqualifying stroke etiology and incomplete data. Cardiac events before CEA were defined as having a past history of myocardial infarction and/or revascularization with coronary artery bypass grafting or percutaneous transluminal coronary angioplasty or percutaneous coronary intervention. Lower extremity peripheral artery disease (PAD) was defined as intermittent claudication and/or revascularization.

Carotid endarterectomy (CEA)

CEA was performed in asymptomatic and symptomatic patients on the basis of current practice guidelines establishing that patients with ≥ 50% carotid artery stenosis and history of ipsilateral stroke or TIA are recommended to have CEA (25-30). Even though patients in the setting of carotid mild stenosis (<50%) are not usually referred for surgery, carotid revascularization was performed based on clinical decision in case of recurrent ischemic cerebrovascular events despite intensive medical therapy including at least one antiplatelet medication. Two senior neurosurgeons (GL, FM) performed all surgical procedures under general anesthesia. Routine intraoperative electroencephalography monitoring for selective intraluminal shunting were used.

Movat Pentachrome staining and carotid IPH

At surgery, the carotid plaque was removed en bloc, immediately fixed in 10% buffered formalin and embedded in paraffin blocks, as previously described (31, 32). Sections at 5-µm thickness were collected on glass slides and stained using Movat Pentachrome staining. Movat stained sections were evaluated for IPH. The digital copies of entire glass slides were created automatically by the Motic Easy Scan (Motic Instruments, Inc., Richmond, BC, Canada) with a 20x objective. IPH was defined as an area of the carotid plaque tissue with blood elements where erythrocytes and fibrin were found extravascular, adjacent to neovessels (2), within the necrotic core or areas of atherosclerotic plaque (3). We excluded the areas related to plaque fissure (ulceration) with consequent extravasation and accumulation of red blood cells and other blood components. Using image analysis software (Aperio's Image Scope software, Leica Biosystems Division of Leica Microsystems, Inc., Buffalo Grove, IL, USA), all Movat stained sections of endarterectomy specimens were blindly reviewed by a cardiovascular pathologist (MCB) (23). The area of IPH and the total plaque area were digitally annotated to generate the extent of IPH (%): [IPH area (µm2) / whole plaque area (µm2)] x 100.

Brain MRI and CMBs

Two blinded neuroradiologists (JCB, LS) reviewed all brain MRIs to assess for the presence or absence of CMBs. Traditionally, T2*-weighted gradient-recall-echo (GRE) is the sequence used to detect CMBs (33, 34). More recently, susceptibility-weighted imaging (SWI), a high-resolution 3D GRE-T2* sequence, has enhanced the visibility and increased the sensitivity for the detection of microbleeds (35-37). For all examinations, either GRE or SWI sequences were evaluated. If both sequences were obtained as part of a single examination, the SWI sequence was used. CMBs were defined as focal, small (less than 10 mm, generally 2-5 mm in diameter) areas of low signal intensity as previously described (11, 17, 38). Both the number and location of each CMB was recorded. Hemorrhage at the site(s) of prior ischemic insults were not included in this analysis, as

they were considered to represent hemorrhagic transformation of an infarct (n=3). Patients with imaging findings concerning for amyloid angiopathy (CMBs generally localized to the gray matter or gray/white junctions, often with evidence of one or more large intraparenchymal hemorrhage, subarachnoid hemorrhage, or superficial siderosis), or hypertensive angiopathy (CMBs generally localized within the basal ganglia and pons) were not included in this analysis, though no such patients were discovered in this cohort. Physiologic calcifications in the basal ganglia, which appear as symmetric hypodensities generally localized to the globi pallidi, were also discounted, as were flow voids related to vascularity.

Computed tomography angiography (CTA)

Severity of carotid artery stenosis was assessed on computed tomography angiography (CTA) as a percentage of lumen narrowing according to the NASCET criteria (29). A single experienced radiologist (JB), blinded to the clinical information and MR brain analysis, performed all the CTA analysis. All examinations were performed on a Siemens single or dual source computed tomography (CT) scanner. The scan range was from the carina through the skull vertex. Scan parameters were set to 120-140 kVp 300-320 mAs for single source, depending on patient habitus, with 75 mm slice reconstruction. For dual source, scan parameters were 100 and 140 kVp and 320 mAs. Both thin slice and reconstructed volumetric data were reviewed. All patients received 100 mL IV injection of Omnipaque 350 followed by a 35ml saline flush.

Statistical analysis

Patients were divided into groups – with or without IPH and with or without CMBs. The absolute and relative frequencies of the categorical data were presented and appropriately analyzed by the Chi-square test or Fisher's exact test. The Kolmogorov–Smirnov test was used to measure the normality of the data distribution for continuous variables. All the non-normally distributed continuous variables were presented as the median and interquartile range (IQR). The continuous variables normally distributed were presented as mean and standard deviation (SD). For between-groups comparisons, the unpaired t-test was used for normally distributed continuous variables, while the Mann–Whitney–Wilcoxon or Kruskal–Wallis test by rank were applied for continuous variables without a normal distribution analysis, where appropriate. Univariate and multivariable logistic regression analysis was performed to test the relationship between carotid IPH extent (%) and the presence of CMBs. The variables considered in the multivariable model were demographic and clinical factors such as age, gender, body mass index (BMI), systolic and diastolic blood pressure, low-density lipoprotein cholesterol (LDL-C), hemoglobin A1c (HbA1c), glucose. For all tests a two-tailed p value < 0.05 was considered statistically significant. The statistical analysis was

performed using IBM SPSS statistical software (SPSS for Windows, release 25.0, SPSS, Chicago, IL, USA) and GraphPad Prism 8.3.0 (GraphPad Software, La Jolla, California, USA).

Results

Carotid IPH and clinical characteristics

In the carotid plaque analysis of the 101 specimens that met the inclusion criteria and were stained with Movat Pentachrome, the prevalence of histologically defined carotid IPH was 57 (53.4%). **Figure 1** shows examples of IPH in carotid artery plaque specimens stained with Movat Pentachrome. The characteristics of the study cohort by IPH presence are shown in **Table 1.** As the results, participants with carotid IPH were more likely male [44 (77.2%) vs 24 (54.5%); p=0.016] and had a higher value of hemoglobin (g/dL) [13.7 (1.6) vs 13.0 (1.4); p=0.022], while those without carotid IPH were more likely presenting with hyperlipidemia [43 (97.7%) vs 48 (84.2%); p=0.024], and with a significantly higher level of total cholesterol [173.0 (152.5-209.5) vs 151.0 (122.2-171.7); p=0.010]. We observed history of ischemic cerebrovascular symptoms in 37 (84.1%) subjects without carotid IPH [stroke: 17 (45.9%); TIA: 15 (40.5%); amaurosis fugax: 5 (13.5%)], and in 49 (86.0%) subjects with carotid IPH [stroke: 32 (65.3%); TIA 10 (20.4%%); amaurosis fugax 7 (14.3%)], without any significant difference between the 2 groups (p=0.793).

CMBs on brain MRI

Brain MRI prior to CEA was available in all the 101 patients. GRE-sequences were analyzed in 86 (85.1%) patients and SWI-sequences in 15 (14.9%). CMBs were reported in 24 (23.7%) patients, with 19 (79.2%) subjects presenting with a single CMB, 5 patients (20.2%) with multiple CMBs (n=1 with 2 CMBs; n=1 with 3 CMBs; n=1 with 5 CMBs; n=1 with 6 CMBs; n=1 with 20 CMBs). The median number of CMBs was 1.0 (1.0-1.0). The localization of CMBs was: temporal (n=3), cerebellum (n=2), parietal (n=3), basal ganglia (n=1), frontal (n=6), 4th ventricle (n=1), subinsular (n=1), multiple (n=4). In 17 (81.0%) patients there was symmetry between the ipsilateral carotid artery and the side of the brain hemisphere with CMBs. The median time interval between brain MR imaging and CEA was 21 (4-103) days, with brain MR imaging preceding CEA in 100% of participants. Examples of CMBs on brain MRI are shown in **Figure 2**.

Carotid IPH and CMBs

Patients with carotid IPH had more CMBs presence compared to patients without IPH [19 (33.3%) vs 5 (11.4%); p=0.010]; **Table 2**. In analyzing the relationship between carotid IPH and CMBs, we also considered the carotid IPH extent (%), that was significantly higher in the carotid plaques of patients with CMBs rather than those in patients without CMBs [9.0 (2.8-27.1) vs 0.9

(0.0-13.9); p=0.004], **Figure 3A**. Moreover, the carotid IPH extent (%) was associated with the number of CMBs (p=0.004), **Figure 3B**. Logistic regression analysis was performed with carotid IPH extent as the independent variable and the presence of CMBs as the dependent variable. This analysis demonstrated the independent association between carotid IPH extent and presence of CMBs. In the univariate analysis, carotid IPH extent was associated to the presence of CMBs [OR 1.051 (95% CI 1.012-1.090); p=0.009]. In the multivariable analysis, carotid IPH extent was associated with presence of CMBs [OR 1.126 (95% CI 1.042-1.217); p=0.003] after adjusting for confounders including age, sex (male), BMI, systolic and diastolic blood pressure, LDL-C, HbA1c, glucose; **Table 3**.

CMBs and degree of carotid artery stenosis

Pre-surgical carotid CTA was available in only 52 (51.5%) patients of the population. The median degree of ipsilateral carotid stenosis was 70 % (50-80 %) in the patients without CMBs and 40 % (35-65 %) in those with CMBs. There was a significant difference between the 2 groups (p=0.049), as shown in **Figure 4**. The group with CMBs had a significantly higher number of patients with carotid stenosis <50% compared with the group without CMBs [5 (71.4%) vs 12 (26.7%; p=0.031].

CMBs and use of antiplatelets

A total number of 86 (85.1%) patients presented with cerebrovascular events prior to CEA. In this group of symptomatic patients, 19 (22 %) had CMBs and 67 (78 %) did not. We noted a statistically significant difference between these 2 groups in the use of antiplatelets at the time of the ischemic cerebrovascular event leading to CEA. Symptomatic patients with presence of CMBs were more on antiplatelets than the symptomatic patients with absence of CMBs [aspirin use: 15 (78.9%) vs 36 (53.7%); p= 0.048, and clopidogrel use: 6 (31.5%) vs 5 (7.4%); p=0.005]. Dual antiplatelet use (aspirin and clopidogrel) was more common in patients with CMBs compared with those without CMBs [6 (40.0%) vs 5 (13.8%); p=0.038], **Table 4**.

Discussion

The results of this study indicate that there is a significant association between carotid artery IPH and CMBs, with both the presence and the amount of IPH in carotid plaque specimens were associated with the presence of CMBs. Additionally, CMBs are associated with a lower degree of carotid stenosis; in symptomatic patients, the presence of CMBs is significantly associated with the use of antiplatelets at the ischemic cerebrovascular event leading to CEA. Overall, these results suggest that CMBs may be in part one of the mechanism of stroke in patients with carotid disease and IPH. Over the last decades, a large consideration has been raised to better understand the

mechanism by which carotid plaque and more specifically nonobstructive carotid plaque is leading and associating with symptoms such as stroke or TIA. We have recently published a study suggesting that IPH may potentially play a role in the mechanism of stroke in patients with nonobstructive carotid stenosis. We extended our research in exploring whether microbleeds in the atheroma of atherosclerotic patients are not exclusively in a single vascular district, but they may be part of a more extended vascular microbleeding, that eventually correlates with cerebrovascular events.

A novelty of our study is that we defined carotid IPH based on the histologically analysis of the surgical specimens. Our idea is that the burden of information captured in the tissue analysis may exceed the limit of the imaging resolution in detecting plaque features under a certain dimension. Consistently with previous studies, for the detection of CMBs on MRI, we used the paramagnetic property of hemosiderin which disrupts the local magnetic field on T2*-weighted GRE sequence (39) and a newer MRI technique, SWI. This sequence has greatly increased the sensitivity of CMBs detection by combining both the magnitude and the phase images to increase susceptibility-related tissue contrast (40).

A growth of interest on CMBs has increased in the last years. However, important issues remain to be clarified in the future on CMBs and their impact on cerebrovascular diseases. It has been suggested that CMBs may be a useful biomarker for pathologic damage to small vessels from hypertension or cerebral amyloid angiopathy (15, 41-45) and a prognostic marker of both future cerebral hemorrhagic (46) and ischemic risk (17, 47, 48). CMBs are more frequent in patients with cerebrovascular diseases and the prevalence is higher in recurrent than first-ever stroke (49). Although a clinical association between the presence of CMBs and ischemic cerebrovascular symptoms was not found in our study, this may be explained by the selection of our population, that included patients undergoing CEA, not the general population of patients with carotid artery disease.

CMBs have been previously associated with carotid artery atherosclerotic disease (24, 50), and with the volume and the presence of specific subcomponents of the carotid plaques (18, 19). Moreover, vulnerable plaques at higher risk for ischemic stroke, defined by both IPH and large lipid-rich necrotic type on MRI, were more frequently observed in patients with CMBs (20). Similarly, our study identifies an association between CMBs and carotid IPH, that is a known factor of plaque vulnerability leading to ischemic cerebrovascular events (1). Our findings add to the previous literature a new insight into the potential relationship of carotid IPH with microbleeds in other vascular beds such as cerebral small vessels in patients with atherosclerosis, especially in those with non-stenotic plaques.

Previous studies have defined CMBs as MRI markers of cerebral small vessel disease as long with white matter hyperintensities, lacunes, and perivascular spaces (22). The total burden of cerebral small vessel disease has been related to carotid IPH and it has been proposed as imaging marker for clinical symptoms in patients with carotid stenosis ≥ 50% (22). Our study differs because it is based on a population of subjects undergoing CEA with any degree of carotid stenosis, including those with carotid stenosis < 50%. Furthermore, there are discrepancies with some studies that did not detect the association between carotid IPH and white matter hyperintensities or lacunes (7, 51), whereas in other studies brain white matter hyperintensities were associated with carotid IPH (52). Thus, it is uncertain whether CMBs may be a useful diagnostic and prognostic marker of small vessel diseases, potentially with therapeutic implications for the treatment of stroke (49). Consistently with previous studies showing the effects of carotid IPH on the cerebral small vessel disease markers (20, 53), our findings confirm that there is an association between carotid artery IPH and CMBs.

IPH instead of the degree of stenosis must be considered as one of the connection between atherosclerotic disease and stroke (7). Additionally, atherosclerosis in one vessel is known to reflect atherosclerotic activity in other vascular beds (54). Thus, carotid plaques are not only related to the ipsilateral ischemic disease. By extension, considering atherosclerosis as a systemic disease, carotid IPH may be a focal manifestation of an underlying generalized process that includes several vulnerable lesions in other vascular territories (55). Whether CMBs are an expression of a systemic disease with shared etiology with carotid IPH or the consequence of a vulnerable plaque with direct and casual link has not established yet.

The association between carotid IPH and CMBs may be explained with a systemic inflammatory response that affects the atherosclerotic carotid arteries along with the cerebral small vessels, which are damaged and allow the bleeding out of the vasculature (56). Potential underlying pathophysiological mechanisms of the development of CMBs may be the release of emboli from carotid IPH into the cerebral vascular system, suggesting that carotid IPH may contribute to the damage of the microstructure of cerebral vessels (52, 57). Accordingly, one of the possible pathogenesis of cerebrovascular ischemic events caused by carotid artery disease may occur through the artery-to-artery embolism (58). The leakage of blood-derived molecules from the small vessels in the cerebral parenchyma promotes an early and persistent peri-lesional inflammation, that is responsible for secondary brain damages such as dendrite degeneration and neuronal death (59, 60).As a matter of fact, inflammatory marker levels and markers of carotid plaque instability are higher in patients with CMBs than those without (61-63). Based on these observations, we may

speculate that inflammatory process is the common underlying mechanism of atherosclerosis and its development in both cerebral small vessels and carotid arteries.

Additionally, our results indicated an association between CMBs and lower degree of carotid artery stenosis. This finding is consistent with our previous study showing that IPH extent in carotid plaque specimens is more associated with mild (<50%) compared to moderate (50-69%) and severe (≥70%) carotid stenosis (23). This may suggest that the early stages of carotid atherosclerosis, before hemodynamically significant degrees of stenosis and severe atherosclerotic plaques development, may be related to hemorrhage-prone small vessel disease (16). Consistently with that, it has been suggested that the progression of common carotid artery atherosclerosis, defined as greater intima-media thickness, may result with lower risk of microhemorrhages represented by CMBs (16).

Although in our study a relationship between CMBs and the use of antiplatelets was demonstrated, there are controversial results suggesting that the presence of CMBs indicates an increased risk of bleeding complications from the use of anticoagulant or antiplatelet drugs. The association may depend on the population studied. In a recent population-based investigation on the relationship between CMBs and antithrombotic medications, anticoagulant use—but not antiplatelet use—was associated with increasing CMB burden (64) and the chance of having at least one cerebral microbleed was predicted by anticoagulant use, male sex and increasing age (64). Conversely, in other studies, antithrombotic use was not associated with CMBs (65) and the use of platelet aggregation inhibitors is related to the presence of CMBs (66-68). In our population, we found the association between CMBs and antiplatelets and while sex was not significantly different in the two groups, with or without CMBs, aging was more associated with the presence of CMBs. Hence, the use of these drugs in guiding therapy decision making remains questionable.

The present study has several limitations, including its relatively small sample size and retrospective design. Some selection bias may be introduced by enrolling the patients who underwent CEA at a single center. Therefore, prospective studies with a larger number of subjects will be required in the future. Furthermore, we have not investigated carotid imaging findings. Since conventional MRI routinely used in clinical practice likely underestimates total CMBs burden (69), possible underestimation of CMBs number may have limited the findings of this study. The use of the available MRI sequence, either GRE or SWI, may have affected the consistency of the imaging analysis, but they are both validated methods used to detect CMBs. Brain MRI has preceded the plaque tissue removal at CEA in all our population, influencing our results. However, because the main aim of our study was not risk prediction but investigating association between carotid IPH and CMBs, we believe that this sequencing will not have affected our results.

Conclusions

In patients undergoing CEA, the histologically defined presence of carotid IPH and its extent are associated with CMBs on brain MR imaging. CMBs may be a potential mechanism for cerebrovascular events in patients with carotid atherosclerotic IPH. Novel indications on the potential value of CMBs for risk stratification in patients with carotid artery disease are provided. CMBs may be a useful imaging marker of the ongoing IPH in atherosclerotic carotid artery. Carotid IPH and CMBs may provide important insights to better understand atherosclerosis as systemic disease and they could be valuable biomarkers. Further studies are needed to investigate the predictive value of carotid IPH and CMBs in patients with carotid artery disease.

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