Using machine learning to identify factors related to nitrous oxide (laughing gas) relapse among adolescents

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INTRODUCTION
Nitrous oxide (N\textsubscript{2}O / laughing gas) has been used in medical practice as an inhalational anaesthetic and analgesic for more than 150 years.\textsuperscript{1} In the past decades, N\textsubscript{2}O exhibited increasing popularity among recreational drug users for its euphoric effects, potentially through its interaction with the endogenous opioid system.\textsuperscript{2} Recreational use of N\textsubscript{2}O emerged as the seventh most used drug globally in the past decade.\textsuperscript{3} The use of N\textsubscript{2}O can cause myelopathy, myocardial injury, anaemia, severe mood disorders, sensory and motor neuropathy, and psychotic symptoms.\textsuperscript{4,5} Prolonged N\textsubscript{2}O intake results in vitamin B\textsubscript{12} deficiency and inhibits methionine synthetase, folate and DNA production, leading to plasma homocysteine (HCY) level elevation and bone marrow haematopoietic dysfunction.\textsuperscript{6} This study aimed to investigate the neurological symptoms of nitrous oxide use and then explore the relapse trajectory and risk factors for relapse.

METHODS
We retrospectively analysed 430 subjects with N\textsubscript{2}O use disorder (averaged 23.90-year-old; 244 men) admitted to a drug rehabilitation programme in Beijing Gaoxin Hospital, with follow-up at 3 and 6 months (from May 2016 to January 2020). The subjects joined a 1-month drug abstinence programme involving psychological consultation and a daily 0.5 mg vitamin B\textsubscript{12} supplement.

The relapse status was obtained from self-report and by confirmation of family members. The basic demographic information (age, gender) and drug use characteristics (total years of regular intake of N\textsubscript{2}O, daily intake dosage of N\textsubscript{2}O) were recorded. Mood disturbances and sleep quality were measured by the Self-Rating Anxiety Scale and the Pittsburgh Sleep Quality Index, respectively.

Craving was measured by Visual Analogue Scales: a line was presented on paper, with the score ‘0’ representing ‘do not want to take N\textsubscript{2}O at all’ and the score ‘100’ representing ‘extreme desire to take N\textsubscript{2}O’. The participants were asked to mark a point on the line which could best represent their craving intensity. A higher score meant higher craving intensity. Peripheral blood samples were obtained at three time points: baseline, 3-month and 6-month follow-up. The biochemical analyses were conducted on the following molecules: aspartic acid transaminase (AST), lactate dehydrogenase (LDH), creatine kinase (CK), α-hydroxybutyrate dehydrogenase (α-HBDH), HCY, vitamin B\textsubscript{12}, folic acid (FA) and mean corpuscular volume (MCV).

The sample sizes of the relapsed and non-relapsed groups were imbalanced to avoid the potential for impairing the classification accuracy. In addition, machine learning provided more accurate alternatives to...
traditional methods, such as Cox regression, in the presence of high-dimensional data. Therefore, we conducted the balanced random forest (BRF) model with the downsampled method to identify factors related to the risks of relapse. The model applied 59 features at baseline to classify relapse conditions at 6 months. We separated the data into the training dataset (70%) and the testing dataset (30%) and tuned the BRF parameters using a 10-fold cross-validation. After any particular feature was permuted across the dataset, the decrease in classification ability was applied to quantify model variable importance. The importance score was scaled between 0 and 100 by subtracting the smallest importance from all observations and dividing it by the most significant importance.

The statistical analyses were conducted in SPSS (IBM SPSS Statistics, V.21) and R Studio (https://www.rstudio.com/) (‘randomForest’ packages) V.7. Student’s independent t-test was applied to compare the baseline differences between the relapsed and non-relapsed groups. Then, Pearson correlation was applied to explore the relationship between biochemical indexes and drug use history. The multiple comparisons were corrected by Bonferroni adjustment.

RESULTS
Of the 430 participants, the mean (sd) years of use were 2.23 (0.64) and the average dosage was 1 796.88±453.92 mL/day. The biochemical indicators AST and HCY suggested myocardial impairment and anaemia (myocardial infarction excluded) in these subjects (online supplemental table 1). Around 55% of individuals had relapsed at 6 months (figure 1M). The anaemia indexes (FA and MCV) and mood disturbances (anxiety and depression), but not myocardial enzymes, showed significant differences between the relapsed group and the non-relapsed group at baseline (FA: t_{428}=3.410, p<0.001; MCV: t_{428}=2.117, p=0.033; anxiety: t_{428}=4.091, p<0.001; depression: t_{428}=2.194, p=0.029) (figure 1A–D; more details can be found in online supplemental table 2). However, after multiple comparisons correction, the MCV and depression did not display significant differences. The myocardial enzymes (AST, LDH, CK and α-HBDH) and HCY, but not anaemia indexes, were positively related to drug use history (dosage and years of addiction) (figure 1E–I), suggesting that the more serious the addiction, the more severe was the myocardial damage. Non-linear regression was applied to fit the individuals’ days to relapse; the data showed log-normal distribution and the number of relapsed individuals peaked at about 40 days (figure 1B).

The model showed medium classification accuracy (area under the curve (receiver operating characteristic curve): 0.685, p=0.009; sensitivity: 0.332; specificity: 0.870), which is reasonable with the limited biochemical indicators and neuropsychological questionnaires included in the model. The model-calculated risk scores for all individuals were obtained (figure 2A,B). The average scores for relapsed and non-relapsed individuals were 0.643 3 and 0.384 9, respectively. Additionally, according to the days to relapse, the risk score for individuals who relapsed before 30 days was higher than for later-relapsed individuals (<30 days: 0.651 7; 30–90 days: 0.630 4; >90 days: 0.618 9).

After any particular feature was permuted across the dataset, the decrease in classification ability was applied to quantify model variable importance. The biochemical and neuropsychological indicators were the most important variables, especially FA, MCV, α-HBDH and anxiety (figure 2D). Individuals with higher FA had less probability of relapse, while individuals with higher LDH, CK, MCV and anxiety scores had a higher probability of relapse (figure 2C). As CK, LDH, α-HBDH and AST constitute the myocardial enzyme spectrum, this suggests that the treatment of myocardial impairment may be of great significance in N2O use disorder recovery.

DISCUSSION
To our knowledge, this is the first report to investigate N2O relapse tendencies and risk factors for relapse. The individuals using N2O demonstrated clear signs of myocardial injury and anaemia; a similar susceptibility to relapse was found when compared with alcohol and opiate patients. The biochemical indicators and mood disturbances at admission are the most important factors in predicting relapse. These findings implicate identifying the clinical population at high risk of relapse and developing effective therapies against N2O use disorder.

Previous studies reported relapse rates of around 50% and 60% for alcohol and opiate patients in 6 months of follow-up, respectively. Our results reported a similar susceptibility to relapse in N2O patients. The relapse distribution is similar to the trend of the incubation of craving: craving increased at 1–3 months and decreased at the later stage of abstinence, suggesting that 1–3 months is a high-risk period for relapse and when more prevention efforts are required.

The results showed that the extent of myocardial injury and anaemia correlated with the years of N2O use and daily dosage, consistent with the previous study that N2O exposure-induced impairment was dose-related. It is believed that a deficiency of the essential FA and serum vitamin B12 can both result in hyperhomocysteinemia, which is associated with an increased risk of cardiovascular diseases. However, in the current study, the individuals with N2O use did not show serum vitamin B12 deficiency, which may be due to self-supplementation of vitamin B12. Moreover, measured serum vitamin B12 levels correspond to extracellular, unbound vitamin B12 which does not reliably reflect intracellular levels. According to the results of the BRF model, FA is the most important factor in relapse prediction, which implies that except for the serum vitamin B12 deficiency,
Figure 1  The relapse pattern and risk factors for individuals with N₂O use disorder. (A–D) Comparison between the relapsed group and non-relapsed group at baseline; (E–L) correlation between biochemical indexes and drug use history; (M) survival curve for time to relapse; (N) non-linear fitting curve for relapse trajectory. * p<0.05; *** p<0.001. α-HBDH, α-hydroxybutyrate dehydrogenase; AST, aspartic acid transaminase; CK, creatine kinase; FA, folic acid; HCY, homocysteine; LDH, lactate dehydrogenase; MCV, mean corpuscular volume; N₂O, nitrous oxide.
the \( \text{N}_2\text{O} \)-caused FA deficiency should get more attention in clinical intervention and relapse prevention.

Mood disturbance is a common comorbidity with substance use disorder.\(^{13}\) Previous studies reported that individuals with \( \text{N}_2\text{O} \) use also commonly have psychiatric symptoms, such as depression, hallucinations and suicidal ideation.\(^{11}\) However, due to the neurological complaints of \( \text{N}_2\text{O} \) use, psychiatric symptoms are often overlooked.\(^{1} \)

Moreover, substance use disorders are commonly associated with poor treatment outcomes, such as higher rates of treatment dropout and relapse.\(^{14,15}\) In the current study, in line with previous findings on substance use disorders, we found that mood disturbance is a vital predictor of relapse in individuals with \( \text{N}_2\text{O} \) use, especially anxiety. These findings suggest that in addition to the regular treatment of neurological disorders caused by \( \text{N}_2\text{O} \) use, providing appropriate medications and psychotherapy may reduce the relapse rate.

Currently, it remains difficult to recognise \( \text{N}_2\text{O} \) use with a urine test or peripheral biomarkers, and a uniform

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**Figure 2** The outcomes of the Balanced Random Forest model. (A) Kernal Density Estimation (KDE) curve for model-calculated risk scores for all individuals; (B) KDE curves for model-calculated risk scores for the non-relapsed group (left) and relapsed group (right); (C) the association between model-calculated risk scores and top 10 most important variables; the black numbers represent insignificant correlation and the red numbers represent significant correlation; (D) scaled importance score for all features. The dark orange indicated the most important variables and the light orange indicated the less important variables. \( \alpha \)-HBDH, \( \alpha \)-hydroxybutyrate dehydrogenase; AST, aspartic acid transaminase; CK, creatine kinase; FA, folic acid; HCY, homocysteine; LDH, lactate dehydrogenase; MCV, mean corpuscular volume; \( \text{N}_2\text{O} \), nitrous oxide.
This finding provides new insights into how to target and manage relapse—clinically relevant biomarkers of relapse. This finding provides new insights into how to target and manage relapse—clinically relevant biomarkers of relapse.

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Correction notice This article has been corrected since it was first published. In the article, the average age of the subjects should be 23.90 years old instead of the “age range 23–90 years”.

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