<u>Screening and referral for brief intervention of alcohol misusing patients in an Accident and</u> <u>Emergency Department: a pragmatic randomised controlled trial.</u>

Short title: Referral for alcohol misuse in an emergency department

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ABSTRACT

Background

Up to a third of patients attending Accident and Emergency (A+E) Departments have consumed excessive alcohol prior to their presentation. The impact of screening and referral for brief intervention by staff working in A+E departments is unknown.

Methods

In a single-blind pragmatic randomised controlled trial, patients were randomised to receive either an information leaflet on alcohol and health or an information leaflet plus an appointment with an Alcohol Health Worker (AHW). Outcome data were collected by patient interview and examination of hospital records at six and 12 months.

Findings

Five hundred and ninety-nine patients were randomised over a 12-month period. At six months, those referred for an appointment with an AHW were consuming a mean of 59.7 units of alcohol per week compared to 83.1 units among those in the control group (t = -2.4, p = 0.02). At twelve months those referred were drinking 57.2 units per week compared to 70.8 in the control arm (t= -1.7, p=0.09). Those referred to the AHW had a mean of 0.5 fewer visits to the A+E over the following 12 months (1.2 compared with 1.7; t= -2.0, p=0.046).

Interpretation

Opportunistic identification and referral for brief intervention for alcohol misuse in an A+E department is feasible, results in lower levels of alcohol consumption over the following six months and reduces reattendance at the A+E. Short-term reductions in alcohol consumption associated with referral for brief intervention for alcohol misuse benefits patients and reduces demand for A+E services.

Key words: Emergency medicine, alcohol misuse, brief intervention, randomised controlled trial

Introduction

Over 14 million people a year are treated in Accident and Emergency (A+E) departments in England. ⁽¹⁾ Given the strong association between alcohol misuse and health related problems such as accidental injury and violence, it is not surprising that the rate of alcohol misuse among people attending A+E departments is higher than that in the general population. As many as one in three attendees have consumed alcohol immediately prior to their presentation and over two-thirds of attendances after midnight may be alcohol related. ⁽²⁾

A person's motivation to reduce their alcohol intake is greater if they are able to make a link between excessive consumption and harm to their health. ⁽³⁾ Such a link may become particularly clear during an attendance at an A+E department. This link, together with high rates of alcohol misuse among attendees, has led to calls for screening for alcohol misuse among people in A+E departments. ⁽⁴⁾ However several important barriers to screening and intervention exist in this setting. These include limited time for managing patients, and staff attitudes to opportunistic identification of alcohol misuse. A+E departments are busy environments with high patient turnover. National requirements to reduce waiting times have added to pressures to treat people as quickly and efficiently as possible, but limits the opportunity to tackle the underlying cause for attendance. ⁽⁵⁾ Identifying and managing alcohol misuse in this environment is therefore a challenging task.

Opportunistic identification followed by brief intervention for alcohol misuse has repeatedly been shown to be effective across a range of medical settings. ⁽⁶⁾ Several attempts have been made to evaluate their impact when offered in A+E departments. Descriptive studies of people offered brief interventions suggest that patients may benefit from such intervention. ⁽⁷⁾ An early attempt to conduct a randomised trial in an A+E department was abandoned due to low levels of screening and uptake of interventions. ⁽⁸⁾ More recent studies have attempted to overcome these problems by deploying trained researchers in an A+E department to screen patients and deliver interventions.

^(9;10) While these studies demonstrated the efficacy of brief interventions in this setting they did not explore their effectiveness. The effect of routine screening and referral by A+E staff has not been investigated in a randomised trial. We therefore set out to examine the impact of this intervention on alcohol consumption, reattendance at the A+E department and quality of life. We used a pragmatic approach to examine the effects of a form of screening and intervention that has been successfully incorporated into routine clinical practice. Findings from an economic evaluation of this intervention are reported in an accompanying paper, ⁽¹¹⁾



Patients and methods

Patients

We conducted a single blind, parallel group, pragmatic randomised controlled trial among patients attending St Mary's A+E department between March 2001 and April 2002. St Mary's Hospital serves an inner-London population of 450,000 residents that are on average younger, more mobile and more ethnically diverse than in other parts of Britain.⁽¹²⁾ Patients were selectively screened for alcohol misuse as part of routine practice in the department, which involves A+E doctors screening patients at the end of the consultation using the Paddington Alcohol Test (PAT). The PAT takes less than a minute to complete and has high sensitivity and specificity compared to longer screening tools for assessing alcohol misuse. ⁽¹³⁾ Any man drinking more than eight units of alcohol in any one session at least once a week, any woman drinking more than six units of alcohol once a week and any person who believes their attendance in the A+E could be related to alcohol is designated 'PAT positive' (i.e. misusing alcohol). ⁽¹⁴⁾ Previous research at St Mary's demonstrated that the number of people who are screened can be increased by targeting those who present with conditions that are most often associated with alcohol misuse. While doctors are encouraged to screen anyone they feel may be consuming excessive alcohol, they are asked to screen all those who present with the following nine conditions: falls, collapse, head injury, assault, gastrointestinal problems, 'unwell', psychiatric problems, cardiac symptoms and accidents. Patients presenting with these problems account for over three-quarters of all alcoholmisusing patients who attend the department. Through the combination of focussed screening and regular audit the department aims to screen over a third of all those who attend. ⁽¹⁵⁾

In addition to being PAT positive, study patients had to be alert and orientated, aged 18 or over, able to speak English sufficiently well to complete study questionnaires and be resident within Greater London. Those already in contact with alcohol services, those already included in the study, and those requesting help with alcohol problems were excluded. All excluded patients were offered an appointment with the Alcohol Health Worker (AHW) as per normal practice. ⁽⁷⁾

Procedures

Those found to be drinking excessively were informed by the A+E doctor that they were consuming alcohol at a level that may be harmful to their health and asked if they would be willing to receive brief intervention. Those who accepted this offer were given written information about the study and asked to provide verbal informed consent. Because patients had limited time to make this decision we attempted to contact all patients during the following seven days to confirm their willingness to take part in the study. Those who said they did not want to take part were excluded from follow up assessments. Local Research Ethics Committee approval was obtained prior to the start of data collection.

Equal numbers of patients were randomised to experimental and control treatment on the basis of randomisation lists derived from a computer program. Opaque envelopes marked with a unique patient identification number were prepared according to the randomisation list. Each envelope contained a copy of a health information leaflet, "*Think About Drink*", ⁽¹⁶⁾ that provides general advice on alcohol including daily benchmark guides for men and women. The leaflet included contact details of national helplines to which we added those for local alcohol support agencies. In addition to the leaflet each envelope also contained either an appointment card asking the patient to reattend for an appointment with the AHW (experimental treatment) or a blank card of the same dimensions and weight as the appointment card (control treatment).

The A+E doctor added the start time and date of the next available appointment with an AHW to the card. AHWs visit the A+E department three mornings a week (Mondays, Wednesdays and Fridays), so the appointment was usually within 36 hours, and always within 72 hours of the patient's presentation to the department. All three AHWs involved in the study were experienced mental health nurses who had undertaken specific training in counselling people who misuse alcohol and had at least five years experience of working with patients with alcohol problems. Those who attended an appointment received approximately 30 minutes of assessment and

discussion of current and previous drinking. AHWs interact with people in a non-confrontational and patient-centred manner. During the course of the assessment patients may resolve ambivalence regarding their drinking and determine appropriate action. However in cases where the patient does not display insight into the consequences of their use of alcohol, the AHW may offer feedback about safe levels of drinking and suggest a range of strategies aimed at reducing levels of consumption. Treatment fidelity was assessed by a researcher not involved in collecting follow up data who examined a random sample of 50 sets of notes made by AHWs. Evidence of assessment of drinking history, current patterns of consumption and information about or referral to other services was determined.

Patient assessment

In order to recruit study patients without impeding the work of A+E doctors we limited collection of baseline data to demographic and clinical details that are collected as part of routine assessment (age, gender, presenting complaint, and data from the PAT). Follow up interviews were conducted either by telephone or in person by a researcher blind to allocation status six months and twelve months after randomisation. At six months we used the Paddington Alcohol Test and Form 90-AQ ⁽¹⁷⁾ to determine alcohol consumption over the previous three months, the General Health Questionnaire ⁽¹⁸⁾ to assess general mental health and a three item questionnaire on suicidal ideation and behaviour. At twelve months we used the PAT, Form 90-AQ, the Time Line Follow Back and the Steady Pattern Grid ⁽¹⁹⁾ to obtain a more detailed measure of alcohol consumption and the EQ-5D ⁽²⁰⁾ to measure of health-related quality of life. Reattendance at the department was examined using local electronic records, and additional data on service utilisation was collected as part of the economic evaluation. ⁽¹¹⁾ Once all other data were collected AHW records were examined to determine whether or not patients had attended an appointment.

Sample size and data analysis

In the absence of data from A+E based trials we used data from the primary care-based study conducted by Wallace and colleagues ⁽²¹⁾ to calculate sample size. In this study 57% received most of the planned intervention, and we estimated that in our study only 45% would do so. We therefore powered our study to be able to examine a proportionately smaller difference in alcohol consumption between groups of 55.6 among controls and 46.2 among those in the experimental group, with standard deviation (SD) of 28.5. A total sample of 388 patients would be required to have 90% power of detecting a difference of this magnitude using a 0.05% level of statistical significance. In anticipation of 30% loss to follow up we increased the sample size to 555.

Baseline data on alcohol consumption, measured using the PAT, and other routine data were used to ascertain whether study groups differed at entry to the trial. We then used data from the Form 90-AQ and Steady Pattern Grid to calculate mean weekly alcohol consumption, drinks per drinking day and percentage days abstinent over a 13-week period measured at 6 months and 12 months. We anticipated that these would not be normally distributed. Despite the skewed distribution of outcome data, we used ordinary parametric tests because this has the advantage of enabling inferences to be made about the arithmetic mean. ⁽²²⁾ Non-parametric bootstrapping was used to assess the robustness of confidence intervals to non-normality of these outcome measures. ⁽²³⁾ Univariate tests were used to examine differences in alcohol consumption between those receiving experimental and control treatment on an intention to treat basis. Regression analysis was then used to adjust for any differences in baseline alcohol consumption or other potential confounding factors. Multivariate models were built using forward stepwise regression. Differences in secondary outcome measures were examined in the same way. Data were analysed using SPSS (version 11.0).

Role of the funding source

The sponsor of the study played no role in the design or conduct of the study or in the writing of this report.

Results

Five thousand two hundred and forty people were screened during the study period of whom 1167 (22.3%) were misusing alcohol (Figure 1). Seven hundred and sixty three of these (65.4%) were willing to accept brief advice, of whom 657 (86.1%) met study inclusion criteria. Most of those who did not meet inclusion criteria either requested to see an AHW or resided outside Greater London. Fifty-eight people refused to take part in the study. The remaining 599 patients (91.2% of eligible patients) were randomised. Four hundred and sixty eight (78.1%) were male and ages ranged from 18 to 90 (mean 44 years). The group reported drinking between three and 94 units of alcohol per session (mean 22 units). Of 440 patients asked whether they thought their attendance in the A+E department was related to alcohol 304 (69.1%) said that it was. Two hundred and eighty seven (47.9%) of the sample were randomised to experimental treatment and 312 (52.1%) to control treatment. Characteristics of those randomised to each arm of the trial are compared in Table 1.

Of the 599 randomised patients, 55 (9.2%) withdrew consent to be contacted for follow up interviews in the week after their entry into the study. At twelve month follow up 384 interviews were completed (64.1% of the randomised patients, 70.6% of those who agreed to be followed up). The rate of follow up in each arm of the trial was similar – 65.8% of those in the experimental arm and 63.5% of those in the control arm. Characteristics of those who were and were not followed up at 12 months are presented in Table 2. Those not followed-up were significantly more likely to believe that their initial A+E attendance was related to alcohol consumption, but no other significant differences were found. In order to test blinding, researchers were asked to predict the randomisation status of a sample of 48 patients after they had completed the 12-month follow up. The correct condition was forecast in 41.6% of cases.

Examination of the records of AHWs showed that 84 (29.3%) of those randomised to the experimental arm of the trial attended an appointment. Of the random sample of AHW notes, 50

(100.0%) detailed current patterns of alcohol consumption, 49 (98.0%) described the patients' drinking history and 41 (82.0%) documented information given and/or referral to other services.

Study outcomes among those who were and were not referred to an AHW are presented in Table 3. The distribution of measures of alcohol consumption at six and 12 months was positively skewed. Log and square root transformation of data were unsuccessful, but comparison findings from non-parametric bootstrapping and parametric t-tests demonstrated the robustness of confidence intervals to non-normality of these outcome measures. At six months those in the experimental arm of the trial were drinking fewer mean units of alcohol per week than those in the control group (t = -2.4, p = 0.02). At twelve months those in the experimental arm of the trial were drinking fewer mean units of alcohol per week than those in the control group (t = -2.4, p = 0.02). At twelve months those in the experimental arm of the trial were still drinking less, but this difference not longer statistically significant (t = -1.7, p = 0.09). Univariate analysis revealed that two other factors were associated with higher levels of mean weekly alcohol consumption at 12 months. Men consumed more alcohol than women (69.1 units compared to 47.9, F=7.4, p = 0.007) and higher levels of baseline consumption, measured by PAT, were associated with higher levels at follow up (r= 0.32, p < 0.001). Inclusion of these factors in a multivariate model did not have a statistically significant effect on the relationship between alcohol consumption and randomisation status.

Those in the experimental arm of the trial made on average 0.5 fewer visits to the A+E department at St Mary's hospital during the year following randomisation. Data from the service utilisation questionnaire revealed that among the 378 for whom data was collected, the mean number of attendances at A+E departments *other* than St Mary's was 0.17 among those in the control arm of the trial and 0.09 among those randomised to ET (t = 1.60, p=0.11). Differences in general mental health and quality of life were not seen.

Data were then reanalysed in order to examine outcome measures among those who did and did not attend an appointment with an AHW. At six months those who attended an appointment were drinking a mean of 14 fewer units of alcohol per week than those who did not attend an

appointment (60.1 units compared to 74.0, F=1.02, p=0.31). No difference in mean weekly alcohol consumption at twelve months was seen among those who attended an appointment with an AHW and those who did not (63.3 units compared to 64.2). The addition of other factors associated with lower alcohol consumption at follow up in a multivariate analysis had little impact on the strength of the association between attendance at an appointment with an AHW and measures of alcohol consumption.



Discussion

This study demonstrated that among people identified as misusing alcohol attending an A+E department, referral for brief intervention from an alcohol health worker was associated with lower alcohol consumption at six months compared to provision of an information leaflet on alcohol and health. Levels of alcohol consumption at six-months were significantly lower among those referred for brief intervention. Levels were also lower in the experimental group at 12 months, but the difference was no longer statistically significant due to a decline in alcohol consumption among controls. This finding contrasts with other studies of brief intervention in which reductions in alcohol consumption have been short lived. ⁽²⁴⁾ The study also confirmed findings of previous work in the department that focussed screening and brief intervention for alcohol misuse is feasible in this setting. ⁽⁷⁾ Lower levels of reattendance in the department were seen among those referred for brief intervention. With a mean reduction of 0.5 visits per person in the experimental group, on average, it would be necessary to treat two people in order to avoid one visit to the A+E department (i.e. for every two people referred for brief intervention one visit to the department over the following 12 months was avoided). Lower levels of alcohol consumption among those referred for brief intervention were not associated with differences in mental health or quality of life.

While current service provision in Britain and elsewhere means that the vast majority of people who misuse alcohol and attend an A+E department receive no special treatment, we considered it unethical to randomise control patients to no intervention in a department where interventions have been offered for over 15 years. ⁽²⁵⁾ Our control patients were provided with a brief intervention that included being told by staff that they were drinking excessive alcohol and being given an information leaflet on alcohol and health that included contact details for local and national alcohol services. Previous research has demonstrated the beneficial effects of health education information on alcohol consumption. ⁽²⁶⁾ By comparing two forms of active intervention we are likely to have underestimated the impact that referral for brief intervention would have

had, if it had been compared to the absence of intervention that constitutes 'treatment as usual' in many other departments.

In this pragmatic trial we aimed to maximise the recruitment of patients by minimising exclusion criteria and using solely clinical staff in the department to recruit patients. While this enabled us to recruit a broad range of patients it meant that we collected limited baseline data and recruited a population that proved difficult to follow up. Herein lie two limitations of the study. First, the limited baseline data that we obtained meant that we were unable to examine changes in outcome measures. While data from baseline PAT provided evidence that alcohol consumption prior to randomisation was similar in each group it is possible that differences in other study outcomes were present at the start of the trial. Secondly, follow up proved difficult. Previous studies in A+E departments have demonstrated that users of emergency services can be difficult to follow up with studies regularly reporting almost half of study patients failing to complete follow up interviews ^(27,7). Our reliance on doctors in the A+E department to recruit patients led to a number of people (n=34, 5.7%) who did not reside in London being inappropriately randomised into the trial. Our decision to seek confirmation of consent to follow up interviews may have further reduced the follow up rate, although we felt this was a necessary step to ensure ethical standards of recruitment. While it is possible that there were differences in the impact of the intervention among those we did not follow up exist, the rate of loss to follow up was similar in each arm of the trial and characteristics of those who did and did not complete the 12 month follow up did not differ significantly.

Although referral for an appointment with an AHW was associated with lower levels of alcohol consumption at six months we did not find a statistically significant reduction in the amount of alcohol consumed by those who attended an appointment compared to those that did not attend. The study was not designed to examine the impact of attendance at an AHW appointment and the

low level of attendance observed meant that we had limited statistical power to examine the impact of seeing an AHW.

In conclusion, screening and referral for brief intervention for alcohol misuse in an A+E department is feasible and results in lower levels of alcohol consumption and reattendance in the emergency department. Attendance at an A+E department provides a 'teachable moment' in which opportunistic identification of alcohol misuse can potentially help patients develop insight into the consequences of their drinking and promote improved health.

(Word Count –3,399)

Contributors: The trial was initiated by M J Crawford and Robin Touquet who, with C Drummond, S Byford and J A Henry, designed the trial. The trial was coordinated by R Patton who, with B Reece, A Brown and B Barrett helped refine study methods and contributed collected study data. R Patton and M J Crawford analysed the data. All authors met regularly as members of the trial steering group and all contributed to trial management. All trial contributors had a role in interpretation of results and approved the final report. M J Crawford is the guarantor.

Conflict of interests: None.

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Figure 1. CONSORT diagram showing patients flow through the study (from screening to 12 month follow up).



Table 1: Baseline characteristics of 599 patients randomised to experimental or control treatment.

| Characteristic | | Control Treatment N=312 | Experimental Treatment N=287 | Difference in means or proportions (95% CI) | |
|------------------------------------------------------------------------------|------------------|----------------------------|------------------------------------|---------------------------------------------------|--|
| Age in years (mean) | | 44.5 | 43.1 | -1.4 (-3.8 to 0.9) | |
| Sex: male (n, %) | | 248 (79.5) | 220 (76.7) | -2.8 (-9.5 to 3.8) | |
| Presenting Condition | Fall | 56 (17.9) | 39 (13.6) | -4.3 (-10.2 to 1.5) | |
| | Collapse | 41 (13.1) | 42 (14.6) | 1.5 (-4.1 to 7.0) | |
| | Head Injury | 12 (3.8) | 21 (7.3) | 3.5 (-0.2 to 7.2) | |
| | Assault | 39 (12.5) | 26 (9.1) | -3.4 (-8.4 to 1.5) | |
| | Gastrointestinal | 39 (12.5) | 34 (11.8) | -0.7 (-5.9 to 4.6) | |
| | Unwell | 35 (11.2) | 48 (16.7) | 5.5 (-0.1 to 11.1) | |
| | Psychiatric | 27 (8.7) | 26 (9.1) | 0.4 (-4.2 to 5.0) | |
| | Cardiac | 23 (7.4) | 19 (6.6) | -0.8 (-4.8 to 3.3) | |
| | Accident | 21 (6.7) | 9 (3.1) | -3.6 (-7.0 to -0.2)* | |
| | Other | 19 (6.1) | 23 (8.0) | 1.9 (-2.2 to 6.0) | |
| Mean units consumed during drinking session | | 20.9 | 21.5 | 0.6 (-1.6 to 2.8) | |
| Believed initial A+E attendance related to drinking [#] (n,%) | | 162 (71.7) | 141 (65.9) | -5.8 (-14.4 to 2.9) | |

p < 0.05, N = 440

| Characteristic | | Followed up 384 (%) | Not followed up 215 (%) | Difference in means or proportions (95% CI) | |
|-----------------------------------------------|------------------|------------------------|----------------------------|---------------------------------------------------|--|
| Age in years (mean) | | 43.9 | 43.7 | -0.2 (-2.3 to 2.6) | |
| Sex: male (n, %) | | 293 (76.3) | 175 (81.4) | 5.1 (-1.6 to 11.8) | |
| Presenting Condition | Fall | 57 (14.8) | 38 (17.7) | 2.9 (-3.4 to 9.1) | |
| | Collapse | 56 (14.6) | 27 (12.6) | -2.0 (-7.7 to 3.6) | |
| | Head Injury | 22 (5.7) | 11 (5.1) | -0.6 (-4.4 to 3.1) | |
| | Assault | 44 (11.5) | 21 (9.8) | -1.7 (-6.8 to 3.4) | |
| | Gastrointestinal | 43 (11.2) | 30 (14.0) | 2.8 (-2.9 to 8.4) | |
| | Unwell | 49 (12.8) | 34 (15.8) | 3.0 (-7.0 to 1.1) | |
| | Psychiatric | 37 (9.6) | 16 (7.4) | -2.2 (-6.8 to 2.4) | |
| | Cardiac | 31 (8.1) | 11 (5.1) | -3.0 (-7.0 to 1.1) | |
| | Accident | 18 (4.7) | 12 (5.6) | 0.9 (-2.8 to 4.6) | |
| | Other | 27 (7.0) | 15 (7.0) | 0.0 (-4.3 to 4.2) | |
| Mean units consumed during drinking | | 21.1 | 21.4 | 0.3 (-2.6 to 2.0) | |
| Believed initial A+E attendance related to | | 190 (65.7) | 113 (74.8) | 9.1 (0.3 to 17.9)* | |
| drinking [#] (n, %) | | | | | |

 Table 2: Baseline characteristics among 599 study patients who were and were not followedup at 12-months

*p < 0.05, [#]N = 440

| Outcome | 6 Months | | | 12 months | | |
|-----------------------------|------------------------------------|-------------------------------|------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------|------------------------------------|
| measure | Experimental Treatment N=174 | Control Treatment N=189 | Difference in means (95% CI) | Experimental Treatment N=189 | Control Treatment N=195 | Difference in means (95% CI) |
| Mean weekly | 59.7 | 83.1 | -23.4 | 57.2 | 70.8 | 13.6 |
| units of consumption | | | (-42.4 to -4 1)* | | | (-29.50 to 2 19) |
| Mean units | 13.0 | 17.1 | -4.1 | 13.1 | 16.0 | 2.9 |
| consumed | | | (-7.2 to | | | (-5.60 to - |
| per drinking dav | | | -1.1)** | | | 0.16)* |
| Percentage | 46.1 | 41.9 | 4.2# | 48.0 | 44.6 | 3.4# |
| Days Abstinent | | | (-3.2 to 11.6) | | | (-3.50 to 10.2) |
| Mean | - | - | - | 1.2 | 1.7 | 0.5 (-1.1 to |
| number of | | | | K | | -0.02)* |
| attendances at local A+E | | | | | | |
| Mean score | 3.7 | 3.4 | -0.25 | - | - | - |
| on GHQ | | | (-1.0 to 0.5) | and the second s | | |
| Mean EQ- | - | | | 0.69 | 0.71 | 0.02 (-0.09 |
| 5D single | | | | | | to 0.05) |
| score | | | | | | |

 Table 3: Alcohol consumption among those in the experimental and control arm of the trial at 6 and 12 months

[#]Difference in proportions, * p <0.05, ** p<0.01.

