**An efficient randomised trial design for multi-cancer screening blood tests: nested enhanced mortality outcomes of screening trial (NEMOST)**

Screening aims to detect cancer at an early, curable stage, and is currently recommended for breast, lung, colorectal, and cervical cancers. Prostate screening may be considered for some men. Individual screening programs for most other cancer types are unlikely to be worthwhile mainly because they each have relatively low incidence and mortality.

Blood tests using next generation sequencing (NGS), based on tumour cell-free nucleic acid or cell-free DNA, can detect signals from multiple cancer types using a single sample; potentially shifting the current approach from 'one test for one cancer' only to the complementary use of 'one test for multiple cancers'.[1,2]

Definitive evaluation of new cancer screening tests has required a randomised controlled trial (RCT) with the primary endpoint cancer-specific mortality.[3] Traditional trial designs involve randomly assigning participants between screening and no screening, then comparing mortality using an intention-to-screen analysis; this approach requires tens of thousands of participants and many follow-up years.

Importantly, clinical benefit does not come from the screening test itself but rather the consequent curative treatment of participants who screened positive. Participants who screened negative receive no special interventions and, because of randomisation, the proportion of these participants is similar in the screened and control groups. Cancer incidence and mortality would also be similar between these two groups. Therefore, the inclusion of deaths among participants who screened negative in the analyses dilutes any effect on mortality. An alternative nested design is borrowed from antenatal screening;[4] which is also used in an ongoing trial of stomach cancer screening[5] (appendix Figure 1). All participants would have blood samples collected but in the control group (non-screened), blood samples only need to assayed in everyone who developed cancer or died and a random proportion of all other controls to confirm that the screen-positive rates are similar in the screened and control groups. This approach cannot be done with imaging screening tests (mammography or low dose CT) because scans in the control group cannot be ignored when they are done.

Using a standard trial analysis, a target relative risk (RR) of 0.85 (460 deaths in the screened group vs 540 deaths in the control group) would probably be a sufficient risk reduction to warrant screening (this target RR is similar to bowel cancer screening); but using the nested design, by focussing on where the clinical benefit of screening actually lies (among those who are positive, whether in the screened group or in the control group), a much lower relative risk could be found (RR 0.70 [190 deaths in those who were positive and screened vs 270 deaths in those who were positive but not screened]); appendix. When only looking at cancers that do not have any current screening (ie, other than breast, bowel, lung, cervix and prostate), a RR of 0.85 requires 218,000 people (80% power) using the standard analysis, but RR 0.70 requires only 96,000 using the nested approach. Not only is the substantial reduction in study size striking, the measure of efficacy avoids the dilution seen with the standard analysis.

Some assumptions that we used to do this analysis, which are listed in the appendix p 2, could change— eg, using projected incidence and deaths for 2021 and later, test sensitivity differs by cancer type, and falsepositive rate lower than 1% yields a positive predictive value of more than 38%. Other sample size methods could be used (eg, using person years), with allowance for non-adherence to annual blood sampling. However, the fundamental principle of a substantial sample size reduction remains.

Cancer-specific mortality could be examined separately for all cancers, all cancers without effective screening, and all cancers that have effective screening tests. The study design should already be powered for analysing stage shift (absolute decrease in advanced stage cancers), representing an initial efficacy analysis. An RCT with advanced cancer stage as the only primary outcome measure is a deviation from using the established screening endpoint of cancer mortality. This approach could lead to equivocal evidence after recruiting many thousands of trial participants.

Participants who screened negative in the screened group might change their behaviour (or not reduce risk factors such as stopping smoking) after being told of their result, thus affecting their cancer risk and mortality. However, evidence so far does not suggest that participants change their behaviour,[6] and lifestyle factors could be measured annually on all participants to check this finding. This potential bias is avoided by masking participants to their trial group allocation, except for those who test positive in the screened group. The nested approach evaluates the direct effect of screening plus curative treatment, whereas the effect of non-adherence to annual testing (which should be similar between groups) would be examined separately, corresponding to efficacy versus effectiveness in screening.[7]

Evaluation of harms must cover the side-effects of invasive biopsies (particularly for identification of benign disease) and treatment modalities, both according to cancer type and the different diagnostic pathways. The screening tests should also show high accuracy (eg, ≥95%) in determining the primary tumour type.

Observational studies indicate promising screening performance of several NGS blood tests, so definitive RCTs are expected, such as for the CancerSEEK (Exact Sciences),[8] Galleri (GRAIL),[9] and PanSeer (Singlera Genomics)[10] tests, and tests originating from the Princess Margaret Cancer Center (Toronto, ON, Canada), and Burning Rock Biotech (Shanghai, China). Investigators could compare design and analytical features between the nested and conventional approaches using their own assumptions (screening performance, incidence, and mortality), alongside feasibility and costs, with a review from screening policy makers. There might also be different types of nested approaches, including a nested analysis within a conventional design (which is not associated with a smaller trial but instead increases statistical power for analysing cancer mortality).

The nested RCT method has key strengths. Cancer mortality can be a primary endpoint without an overly large, long, and expensive trial. Controversy over the value of using advanced cancer as the only primary endpoint is avoided. Given that there are different multi-cancer tests, two or more RCTs could be done in a reasonable timeframe. A new approach for evaluating early cancer detection is therefore feasible.

Professor Allan Hackshaw, MSc
Cancer Research UK & UCL Cancer Trials Centre
University College London
London
UK

Professor Christine D. Berg, MD
Formerly, Division of Cancer Prevention
US National Cancer Institute
Bethesda, Maryland
USA

**References**

1. Ahlquist DA. Universal cancer screening: revolutionary, rational, and realizable. npj Precision Onc 2018; 2: 23.

2 Liu MC. Transforming the landscape of early cancer detection using blood tests-commentary on current methodologies and future prospects. Br J Cancer 2021; published online Feb 9. https://doi.org/10.1038/ s41416-020-01223-7.

3 Eeles RA, Berg CD, Tobias JS, eds. Cancer prevention and screening: concepts, principles and controversies. Hoboken, NJ: Wiley-Blackwell, 2018.

4 Lazarus JH, Bestwick JP, Channon S, et al. Antenatal thyroid screening and childhood cognitive function. N Engl J Med 2012; 366: 493–501.

5 Wald NJ. The treatment of Helicobacter pylori infection of the stomach in relation to the possible prevention of gastric cancer. In: Helicobacter pylori eradication as a strategy for preventing gastric cancer. Lyon, France: International Agency for Research on Cancer. IARC Working Group Reports, 2014: 174–80.

6 Cooper CC, Harvie MN, French DP. Do negative screening test results cause false reassurance? A systematic review. Br J Health Psychol 2017; 22: 958–77.

7 Wald NJ. Efficacy and effectiveness. J Med Screen 2021; published online Feb 20. https://doi.org/10.1177/0969141321995223.

8 Lennon AM, Buchanan AH, Kinde I, et al. Feasibility of blood testing combined with PET-CT to screen for cancer and guide intervention. Science 2020; 369: eabb9601.

9 NHS. NHS to pilot potentially revolutionary blood test that detects more than 50 cancers. 2020. https://www.england.nhs.uk/2020/11/nhs-topilot-potentially-revolutionary-blood-test/ (accessed April 13, 2021).

10 Chen X, Gole J, Gore A, et al. Non-invasive early detection of cancer four years before conventional diagnosis using a blood test. Nat Commun 2020; 11: 347