Trial of management of borderline and other low-grade abnormal smears (TOMBOLA): Trial design☆

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Abstract

Cervical screening reduces the risk of cervical cancer by detecting and treating cervical intraepithelial neoplasia (CIN). The management of women with low-grade cervical abnormalities is controversial. Two management policies exist: repeat smears in primary care and colposcopy examination. It is not clear which of these is the more effective and efficient. There is also uncertainty as to the most effective and efficient management of women at colposcopy when an area of abnormality is seen on the cervix — immediate treatment or biopsy and selective recall for treatment if the biopsy result suggests this is necessary. The result of a human papillomavirus (HPV) test might assist in deciding the appropriate management of women with low-grade abnormalities. TOMBOLA, a pragmatic randomised-controlled trial set within the cervical screening programmes in Scotland and England, addresses these three areas of uncertainty. Almost four and a half thousand women aged 20–59 with a low-grade cervical abnormality have been recruited and randomised to either repeat smears or colposcopy examination. Women in the colposcopy arm of the trial are further randomised to a policy of either immediate treatment or biopsy and selective recall for treatment if they have an abnormal transformation zone. Women are followed up to an exit examination at 3 years. HPV testing is undertaken at recruitment and at the exit examination. The primary endpoint is cumulative incidence of CIN2/3. A range of other clinical, psychosocial and economic outcomes is being considered. This paper describes the design of the trial, and discusses the rationale underlying aspects of the design and the challenges faced in designing and implementing the trial.

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Keywords: Randomised-controlled trial; Cervical screening; Colposcopy; Cytological surveillance; Management; Low-grade abnormalities; Cervix uteri; Mild dyskaryosis

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1. Introduction

By detecting and treating cervical intraepithelial neoplasia (CIN), cervical screening has reduced the incidence of, and mortality due to, cervical cancer. In the UK, screening is based on 3–5 yearly cytological smear tests for women aged 20 or 25 to 60 or 65. Smears are classified as “unsatisfactory”, “normal”, “borderline nuclear abnormalities” (BNA), “mild”, “moderate” or “severe dyskaryosis” or “indicating invasive cancer”. Most clinicians consider smears showing BNA or mild dyskaryosis as “low-grade” and those showing moderate or severe dyskaryosis as “high-grade”. There are well-established guidelines for management of women with high-grade abnormalities but the management of women with low-grade abnormalities is controversial. Each year in the UK, more than 250,000 cytological smears show low-grade abnormalities [1,2], so this uncertainty regarding management has important implications for both women and the National Health Service.

Two methods of management for women with low-grade abnormalities are routinely employed — repeat smears in primary care at regular intervals (cytological surveillance) or colposcopic examination in a hospital outpatient clinic, with biopsy if an abnormal area is seen on the cervix. It is not clear which of these is the more effective or efficient management policy. Revised guidelines on colposcopy and programme management for the NHS Cervical Screening Programme in England reflect this uncertainty, suggesting that “women should be referred for colposcopy after one test reported as mild dyskaryosis, but it is acceptable to recommend a repeat test” [3]. Referral to colposcopy after a single mild smear will increase the number of referrals to colposcopy, which may increase waiting times. In Scotland, there has been reluctance to deviate from the previous guidelines which applied to the whole of the UK, deferring colposcopy until two smears showing mild dyskaryosis or three smears showing BNA had been reported [4]. In addition, there is considerable heterogeneity in management across the UK. Furthermore, over time and without treatment, some low-grade lesions will progress to higher-grade lesions, others will remain stable, and others will regress spontaneously; follow-up and treatment may therefore be of no benefit to the group of women who have a low-grade lesion that is going to regress. At the moment, however, it is not possible to identify which abnormalities will regress, and which will progress.

Human papillomavirus (HPV) has been shown to have a role in the aetiology of cervical cancer and pre-cancer, with certain types leading to a particularly high risk [5,6]. This has led to suggestions that HPV testing would be of potential value in the triage of women with low-grade abnormalities [7,8]. If HPV testing could be used to discriminate between women with a low-grade smear who are likely to have underlying high-grade CIN and those who are unlikely to have high-grade disease, then it might be possible to manage the two groups of women in different ways. For example, those likely to have underlying disease might be referred straight to colposcopy, while those unlikely to have underlying disease could be kept under cytological surveillance, thus reducing the burden on colposcopy clinics in terms of new referrals.

There is also uncertainty as to the most effective and efficient method of managing women with a low-grade smear when an area of abnormality is seen on the cervix during colposcopy. There are two main methods — biopsy followed by recall for treatment if the histological result confirms CIN or immediate treatment [9]. The recent English guidelines discourage immediate treatment for women with low-grade smears, because of the potential for over-treatment [3]. However, trial evidence is lacking on whether immediate treatment or biopsy and recall is the most effective and efficient policy.

In 1995 the Medical Research Council and Department of Health issued a joint commissioning brief for a randomised-controlled trial of alternative referral and management policies for women with low-grade smears, to include a HPV component (BMJ, 10 June 1995, vol 310). TOMBOLA (Trial Of Management of Borderline and Other Low-grade Abnormal smears) is the result of that call.

This paper describes the design of TOMBOLA, the outcomes being considered, and discusses some of the issues arising in the design and implementation of this pragmatic trial nested within the NHS Cervical Screening Programmes in England and Scotland.

2. Aims

The aims of TOMBOLA are, in women with a low-grade smear:

- To determine whether cytological surveillance or a colposcopic examination is the more effective and efficient management policy;
In those in whom an abnormal transformation zone is noted during colposcopy, to determine whether immediate
treatment (using large loop excision of the transformation zone; LLETZ) or biopsies and recall if necessary for
LLETZ is the more effective and efficient treatment policy;
To evaluate the contribution of HPV testing to these policies.

3. Methods

3.1. Trial design

TOMBOLA is a pragmatic randomised-controlled trial nested within the Cervical Screening Programmes in two
areas of Scotland (Grampian and Tayside) and one area of England (Nottingham). Fig. 1 shows the trial design. Eligible
women were recruited through hospital-based clinics. A swab sample from the cervix was taken at recruitment for HPV
testing. Women were classified as high-risk HPV positive if they tested positive for any of 14 high-risk HPV types, or
high-risk HPV negative if they did not test positive for any of these high-risk types. Women were randomised to a
policy of either cytological surveillance or hospital-based colposcopy.

Management of women in the trial is not based on their HPV status. This is to avoid confounding of management
and HPV status. Instead, interactions between HPV and management will be assessed. If colposcopy and cytological
surveillance were found to be equally effective in high-risk HPV positive women and in high-risk HPV negative
women, this would imply that HPV testing would not be an effective adjunct to the cervical screening protocol for the
purpose of triage. If colposcopy were more effective than cytological surveillance, or vice versa, in (i) women who
were high-risk HPV positive, but not in those who were high-risk HPV negative, or in (ii) women who were high-risk
HPV negative, but not in those who were high-risk HPV positive, this would imply that addition of HPV testing would
increase the clinical effectiveness of the cervical screening protocol.

Women who were randomised to colposcopy were further randomised to a policy of immediate treatment or biopsy
and selective recall for treatment. Again, women’s treatment was not based on their HPV status, which enables
assessment of interactions between HPV and treatment options.

Women are followed-up for three years after recruitment to an exit appointment where a colposcopic examination is
undertaken and another sample for HPV testing obtained.

The decisions to fund the trial were taken in 1997/1998. Recruitment began in 1999 and continued to January 2003.
Exit clinics began in January 2003 and were completed in December 2005. Final results will be available in 2006/2007.

3.2. Ethical approval

Prior to the start of the trial, ethical approval was obtained from the local ethics committees in each of the study
areas. The General Practice sub-committees in each area were also consulted about the trial.

3.3. Eligibility criteria

There were two phases of recruitment (Table 1). Throughout the recruitment period (ie in both phases), women were
potentially eligible if they:

(i) had an “index” smear showing a low-grade abnormality — either mild dyskaryosis or BNA.
(ii) were resident in the Grampian Health Board area, Tayside Health Board area or in the Nottingham area with their
index smear processed by the Aberdeen, Dundee or Nottingham Cytology Laboratories. The Aberdeen and
Dundee Cytology Laboratories process all smears for residents in Grampian and Tayside Health Board areas, the
Nottingham Cytology Laboratory processes smears from Nottingham city and surrounding areas;
(iii) were aged 20–59 at the date of the index smear;
(iv) were not pregnant at the time of recruitment; and
(v) had not had previous destructive or excisional treatment for proven or suspected cervical lesions.

Additional, but different, eligibility criteria were in operation during the two phases of recruitment. In the first
recruitment phase women were eligible if they had had no abnormal smears in the previous three years and they (i) had
an index smear showing mild dyskaryosis or (ii) had an index smear showing BNA with a repeat smear showing mild dyskaryosis or BNA at the TOMBOLA recruitment clinic 6 months later. The first phase of recruitment ran from October 1999 to March 2001.
The first phase of recruitment was designed to address concern about potential over-treatment of women with BNA. For this reason, women with an index smear showing BNA were only eligible if they had a subsequent smear, approximately 6 months after the index smear, that showed a further low-grade abnormality. Having two BNA smears 6 months apart was taken to indicate a persistent low-grade abnormality. At the time the trial was funded, the occurrence of a single smear showing BNA was not considered to be clinically important and the NHS CSP guidelines discouraged active treatment of these women [10]. Indeed the guidelines suggested referral to colposcopy only after 3 smears, 6 months apart, showing BNA.

In the second phase of recruitment, women were eligible if they had an index smear showing either mild dyskaryosis or BNA and in the previous three years had had either no abnormal smears or one smear showing BNA, (ie women with a single BNA (or mild) smear were eligible). The second phase of recruitment ran from March 2001 to January 2003.

The decision to change the eligibility criteria relating to the smear status was the result of four factors. First, since the original commissioning brief, the climate of opinion regarding the management of women with low-grade abnormal smears appeared to have changed. While there was little new scientific evidence on whether referring women with a single BNA smear to colposcopy was necessary, or whether this simply led to over-treatment, there were a number of court cases relating to the cervical screening programmes. Anecdotally, there seemed to be a more interventionist approach to the management of women with low-grade abnormalities, with referral to colposcopy occurring after more minor abnormalities, and this appeared to be the result of the threat of litigation. In addition, new studies had been funded where women who presented with a single BNA smear (who were HPV positive) were referred for colposcopy, and possibly related interventions [11–13]. The TOMBOLA group, therefore, considered that the inclusion of women with a single BNA smear in TOMBOLA might represent a last opportunity to determine the most effective and efficient management for these women, and potentially to conclude that such interventionist management is unnecessary for the majority. Second, it was postulated that women with a single BNA smear might be the very group for whom HPV testing might be most valuable in triage, and that a randomised-controlled trial would provide useful evidence on the value, if any, of HPV triage of these women. Third, under the original eligibility criteria, a large proportion of women with an index smear showing BNA were not eligible on the basis of the result of their recruitment smear [14]. Sixty-three percent of women with an index smear showing BNA who consented to take part in TOMBOLA were not eligible on the basis of their recruitment smear, the majority having a negative recruitment smear, and a small proportion a recruitment smear showing moderate dyskaryosis or worse. Women who were not eligible on the basis of their recruitment smear had undergone the recruitment process, and the time involved in this impacted on the women themselves, and the workload of trial staff. Finally, lower numbers of eligible women were identified than originally anticipated. In the planning stages of the trial, woman-based screening profiles in the trial areas were not available and projected recruitment had been based on numbers of smears, with clinical experience used to make estimates of numbers of women. This exercise resulted in an overestimation of the potential study population. Altering the eligibility criteria increased the numbers of eligible women. The MRC and local ethics committees approved the change in eligibility and the second phase of recruitment was implemented in March 2001.

### 3.4. Recruitment

Cervical screening in the UK is organised regionally. The majority of smears are taken in primary care, with a smaller number through family planning, genito-urinary and other clinics. Within each region, smears are read centrally.
in the cytopathology laboratory for that area, and the cytopathology laboratories maintain databases of smear results. Smear results are reported to women either directly from the laboratory or via their general practitioner.

Prior to beginning recruitment, GPs in the three study areas were invited to presentations about the trial. They were also sent information packs and offered a practice visit by a member of TOMBOLA staff. GP practices were given the opportunity to “opt-out” of the trial, and if they did so, none of their patients would have been invited to take part. One GP practice opted out.

Eligible women were identified from the computerised records held at the three relevant cytopathology laboratories (in Aberdeen, Dundee and Nottingham) and their details were downloaded into a dedicated study database. A letter was then sent to the GP of each eligible woman informing them that we proposed to invite their patient to participate in TOMBOLA. This gave GPs an opportunity to “opt-out” individual women from the trial. We were asked not to approach around 30 individual women, primarily because they had other medical conditions, language difficulties or difficult personal circumstances.

An information leaflet was sent to each eligible woman together with an appointment to attend a TOMBOLA recruitment clinic. Recruitment clinics were held in Aberdeen (Aberdeen Royal Infirmary), Elgin (Dr Gray’s Hospital), Dundee (Ninewells Hospital), Perth (Perth Royal Infirmary) and Nottingham (Queens Medical Centre and City Hospital). Women were provided with a freephone contact telephone number for their local study centre (Aberdeen, Dundee and Nottingham). If the appointment was not convenient, women were invited to telephone the study freephone to re-schedule their appointment. They were also invited to call the freephone for further information about the trial.

At the recruitment appointment, a research nurse explained the protocol to the woman and sought informed consent. Women who consented were asked to complete questionnaires on socio-demographic and life-style factors and psychosocial characteristics (more information is provided on these questionnaires in the Outcomes section below). A sample for HPV testing was also sought, and taken as an endo-cervical sample with a cytobrush which was immersed into 2 ml of sterile phosphate buffered saline containing 0.05% thiomersal. All samples were analysed at the HPV laboratory in Nottingham. Women were told that the sample was for “additional testing” rather than for HPV testing. In planning the trial both disclosure and non-disclosure of HPV results were considered. For a number of reasons, it was agreed to not disclose the nature of the test, or the results of the test. First, the significance of HPV infection in women with low-grade smears is unclear, and disclosure of results of unproven clinical significance could cause difficulty and confusion both to women and to clinicians. In addition, the information may have caused women distress and anxiety. Second, for scientific reasons (ie avoidance of confounding) management decisions within the trial were not based on the HPV result. This approach was approved by the local ethics committees in each of the study areas. Less than 1% of women asked specifically about the “additional testing”, and they were told that the sample was for HPV testing for research purposes, and that the result would not be used in their management or disclosed to their clinicians or themselves. Some women (less than 10%) consented to take part in the trial and were recruited, but did not provide an HPV sample at recruitment; in most cases this was because they were menstruating.

In the first recruitment phase, women with a borderline index smear also had a smear taken at their recruitment appointment. If this smear showed BNA or mild dyskaryosis they were eligible for recruitment into TOMBOLA and went on to be randomised. If the smear was normal, or showed moderate dyskaryosis or worse, the woman was not eligible for TOMBOLA. These women were followed-up appropriately under the NHS clinical protocol used routinely in the local area; this meant follow-up by repeat cytology for women with a normal smear or referral for colposcopy for those with a smear showing moderate dyskaryosis or worse.

At the end of the recruitment appointment, women were told that they would be informed, by post, of their randomisation, in a few weeks’ time. This interval was to enable HPV testing to be undertaken prior to randomisation (see Randomisation 1 (R1)). In the first phase of recruitment, it also enabled the recruitment smear to be read and reported.

If a woman attended the recruitment clinic but did not consent to take part in TOMBOLA she was returned to the care of her GP to be managed under the local NHS protocol for that area, by either repeat cytology or referral for colposcopy. If a woman did not attend the first appointment that was offered to her at the TOMBOLA recruitment clinic, she was offered a second appointment. If she did not attend this second appointment, the woman was not approached again and was referred back to the care of her GP and subsequently managed under the local NHS protocol for that area, by either repeat cytology or referral for colposcopy.

3.5. HPV testing

Samples for HPV testing were taken from women at recruitment, and at their exit examination at the end of the 3-year follow-up (see section on Exit examination below). The woman’s HPV status at each time-point was
determined. Human and viral DNA was extracted from the swabs using a kit purchased from Qiagen UK (QIAamp® DNA Mini Kit) using the manufacturer’s protocol optimised by the authors. Negative controls containing only buffer (elution buffer AE) were included in each batch. The extracted DNA was amplified and quantitated using a type-specific real-time PCR for a housekeeping gene, human betaglobin. Samples without recordable levels of DNA were classified as inadequate. Samples with recordable levels of DNA were subjected to HPV PCR using the consensus primers GP5+/6+ followed by Enzyme Immunoassay for the detection of 14 high-risk HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68) [15]. Women were classified as high-risk HPV positive if their sample had an optical density (OD) reading of 3 times greater than that of the OD of the negative controls included in each assay, implying that they carried one or more of these strains of high-risk HPV genotype. Women whose samples did not test positive on this assay were classified as high-risk HPV negative. Less than 1% of the recruitment HPV samples were inadequate for analysis. The result of the HPV testing at recruitment was used to stratify randomisation.

3.6. Randomisation 1 (R1)

The first randomisation was to either cytological surveillance in primary care or hospital-based colposcopy. Balanced randomisation was used in that 50% of women were randomised to each arm. The first randomisation was undertaken when the results of the recruitment HPV sample were received, between one and four weeks after recruitment. Randomisation was undertaken using a dedicated touch-tone telephone randomisation service provided by the Health Services Research Unit of Aberdeen University. Randomisation was stratified by age-group, index smear, HPV result and recruitment centre (Table 2) to ensure that factors were balanced between the trial arms. Women who were recruited into TOMBOLA without an HPV sample being taken at recruitment were classified as “no consent for HPV/no HPV sample available” for the purposes of randomisation.

3.7. Cytological surveillance

Cytological surveillance involves repeat smears at six-monthly intervals in primary care. Women randomised to cytological surveillance were notified of their randomisation, by post, and informed when their next smear was due. Approximately one month before the first smear in surveillance was due, a “trigger letter” was sent by TOMBOLA to

<table>
<thead>
<tr>
<th>Stratification variable</th>
<th>Stratification categories</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age group</td>
<td>20–29 years, 30–39 years, 40–49 years, 50–59 years</td>
</tr>
<tr>
<td>Index smear</td>
<td>In first recruitment phase (Mild index, BNA index with mild repeat smear, BNA index with BNA repeat smear) In second recruitment phase (Mild index with no history of abnormal smear, Mild index with history of previous BNA smear, BNA index with no history of previous abnormal smear, BNA index with history of previous BNA smear)</td>
</tr>
<tr>
<td>HPV result</td>
<td>High-risk HPV positive, High-risk HPV negative, Inadequate sample for analysis, No consent for HPV/no HPV sample available</td>
</tr>
<tr>
<td>Recruitment centre</td>
<td>Grampian, Tayside, Nottingham</td>
</tr>
</tbody>
</table>

a These are equivalent groups from first and second recruitment phases.

b BNA, Borderline Nuclear Abnormalities.
both the woman and her GP, reminding them that it was due. Trigger letters were also sent for subsequent smears to remind women and GPs that they were due. If a woman became three months or more overdue for her smear, a reminder letter was sent informing her of this.

The first smear in surveillance was due six months after the woman’s index or recruitment smear. Her second smear in surveillance would be due six months after the first was taken; and the third due six months after the second was taken, and so on.

The date and result of all smears taken in primary care are imported directly from the computerised records at the three relevant cytopathology laboratories into the dedicated TOMBOLA database. In the Nottingham centre, results of smears from participants in TOMBOLA are linked with their record in the TOMBOLA database using date-of-birth and name. In the Scottish centres this linkage is based on the Community Health Index (CHI) number, which is unique for everyone registered with a GP in Scotland [16].

If a woman has three consecutive normal smears, she is returned to “routine recall”, ie smears at 3-yearly intervals within the screening programme. If a woman has a smear that shows moderate dyskaryosis or worse, or three consecutive inadequate smears, referral is made for a colposcopy at an NHS clinic, as per the screening programme guidelines [3,4]. Otherwise, she remains on 6-monthly recall for smears (see Table 3).

3.8. Hospital-based colposcopy and second randomisation (R2)

At a hospital-based colposcopy appointment, the colposcopist visualises the cervix to identify any areas of abnormality. Women randomised to colposcopy were informed, by post, of their randomisation, and a colposcopy appointment was issued. Information about colposcopy and the second randomisation – to either biopsy and selective recall for treatment (if needed, based on the biopsy result) or immediate treatment by LLETZ – was enclosed. At the colposcopy appointment, a research nurse sought additional consent from the woman for the second randomisation.

If consent was obtained, the second randomisation was done immediately by the research nurse using the dedicated randomisation service provided by the Health Services Research Unit at Aberdeen University. The same stratification criteria were used as for the first randomisation (see Table 2). Balanced randomisation was again used in that 50% of women were randomised each arm.

3.8.1. Women who consented to the second randomisation

The colposcopy examination was undertaken. If an abnormal transformation zone was seen and the upper limit could be identified, the management policy to which the woman was randomised was followed immediately.

Biopsy and selective recall involved taking a minimum of two targeted punch biopsies from the areas that colposcopically looked most abnormal. These were sent to the local histopathology laboratory for light microscopy, the report usually being available within 10 days. If the histology result showed no CIN or CIN1 no further treatment was required. However, if the biopsy histology showed CIN2/3 the woman was recalled to the TOMBOLA clinic for treatment by LLETZ. The excised tissue was sent to the local histopathology laboratory for light microscopy.

Table 3

<table>
<thead>
<tr>
<th>Action following reporting of smear results</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Women in the cytological surveillance arm</strong></td>
<td></td>
</tr>
<tr>
<td>3 consecutive normal smears</td>
<td>Return to routine three-yearly screening</td>
</tr>
<tr>
<td>Continued low-grade smears</td>
<td>Continue with six-monthly repeat smears</td>
</tr>
<tr>
<td>A smear that shows moderate dyskaryosis or worse</td>
<td>Referral for an NHS colposcopy and follow-up as required</td>
</tr>
<tr>
<td>3 consecutive inadequate smears</td>
<td>Referral for NHS colposcopy and follow-up as required</td>
</tr>
<tr>
<td><strong>Women in the colposcopy arm</strong></td>
<td></td>
</tr>
<tr>
<td>3 consecutive normal smears</td>
<td>Return to routine three-yearly screening</td>
</tr>
</tbody>
</table>
| Continued low-grade smears | Continue with six-monthly repeat smears or re-referral to colposcopy

\[a\] The action in this situation is determined by the colposcopy and any histology results.
If the woman was randomised to immediate treatment, the whole of the transformation zone, including the area(s) that appeared abnormal was removed by LLETZ during the colposcopy appointment using LLETZ and the excised tissue sent to the local histopathology laboratory for light microscopy. If the transformation zone appeared normal during the colposcopy, the woman did not undergo any additional treatment or procedures at that time. If the squamo-columnar junction could not be visualised, the woman was treated according to local NHS protocols.

The colposcopy results, and the histology of any biopsy and LLETZ material was reported to both the woman and her GP by TOMBOLA, together with recommendations for follow-up after colposcopy. For the majority of women, follow-up after colposcopy in the TOMBOLA clinic is by regular smears, the results of which are captured into the TOMBOLA database. Consistent with standard clinical practice, annual or six-monthly smears are recommended, depending on the results of the colposcopy and any histology. The smear results are monitored, and subsequent action (in terms of recommending next smear date or referral to colposcopy) is based on these results (see Table 3 for details). Follow-up after colposcopy where the biopsy or LLETZ histology was worse than CIN3, or where there was an indication of involved margins is according to local NHS protocol.

3.8.2. Women who did not consent to the second randomisation

The colposcopy was undertaken and the woman treated according to local NHS protocol for that area. These women remain in the trial and will be invited to their exit examination (see below) at the end of their three-year follow-up.

3.8.3. Women who did not attend their colposcopy appointment

If a woman did not attend the first appointment that was offered to her at the TOMBOLA colposcopy clinic, she was offered a second appointment. If she did not attend this second appointment, the woman was managed according to the local NHS protocol. In order to avoid introducing bias, these women remain in the trial and will be invited to their exit examination at the end of the three-year follow-up, and will be included in the analyses on the basis of intention-to-treat. Similarly if a woman failed to return for a treatment appointment, she was managed according to the local NHS protocol. She will be invited to an exit examination and included in the analyses on the basis of intention-to-treat.

3.9. Follow-up

After recruitment, women are followed-up for three years. Questionnaire data are collected from women during this period (described in the Outcomes section below).

If a woman becomes pregnant after recruitment, her management is temporarily suspended for the duration of her pregnancy, as would be standard practice outwith the trial. When the pregnancy ends, her management recommences. For example, any smear that became due during pregnancy would be rescheduled after the end of the pregnancy. In the colposcopy arm, a few women became pregnant between recruitment and their colposcopy appointment; in such cases, the woman underwent colposcopy but any treatment was deferred until the end of their pregnancy.

3.10. Exit examination

Approximately three years after recruitment into the trial women are invited back to the TOMBOLA clinic for an “exit examination”. At the exit examination, a sample for HPV testing is obtained. A colposcopic examination is also undertaken, with treatment using LLETZ if an abnormal transformation zone is seen.

During the colposcopic examination, the clinician is blinded to the woman’s initial smear status, her randomisation, and to any clinical outcomes (both cytology and histology) that occurred during the three-year follow-up period. The purpose of this blinding is to guard against potential bias in the assessment of whether a woman has an abnormal transformation zone or not. After the colposcopist has viewed the cervix and recorded their colposcopic impression, they have access to the woman’s records and notes to inform decisions about any treatment and subsequent follow-up and recall by the NHS Cervical Screening Programme.
After a woman’s exit examination is complete, and any histology reported, a letter is sent to her and her GP reporting the results and informing them of her recommended follow-up, which is either colposcopy in an NHS clinic or smears in primary care.

3.11. Deviation from trial protocol

Deviation from protocol is noted on occasions where a woman does not follow the management procedure to which she was randomised. Women who deviate from the trial protocol are followed up outwith TOMBOLA according to local NHS protocol. The numbers of women who have deviated from trial protocol are small. Examples of deviation from protocol include:

- women who are randomised to cytological surveillance but who are referred to colposcopy by their GP for reasons other than a smear showing moderate dyskaryosis or worse or 3 inadequate smears;
- women randomised to immediate LLETZ where biopsies are taken rather than a LLETZ; and
- women randomised to biopsy and selective recall where a LLETZ is undertaken rather than biopsies.

All women who deviate from trial protocol will be invited for an exit examination at the usual time, and will be included in the analysis by intention-to-treat (see Analysis below).

4. Outcomes

A range of clinical, psychosocial and health economic outcomes are being considered within TOMBOLA. These are summarised in Table 4. Table 5(a) and (b) describes the timing of data collection in relation to (a) clinical outcomes and (b) other outcomes ascertained by questionnaire.

4.1. Clinical outcomes

4.1.1. CIN2/3

The primary clinical outcome is the cumulative incidence of CIN2, CIN3 or more severe disease (henceforth CIN2/3) over the period from recruitment up to, and including, the three-year exit examination. CIN2/3 has been chosen as the primary clinical outcome as it represents a pre-malignant stage of cervical lesions. In order to reduce morbidity and mortality due to invasive cervical cancer, the cervical screening programmes aim to detect and treat pre-malignant cervical lesions. In this context, CIN2/3 is the most appropriate primary endpoint for the trial, and is widely accepted as a surrogate endpoint in cervical screening research.

Information on CIN is recorded at TOMBOLA clinic appointments. Additional information from non-TOMBOLA appointments is being obtained from hospital records and pathology databases. Around the time of the exit appointment, a woman’s medical records, together with the hospital and pathology databases, are interrogated to ascertain details of any additional procedures relating to the woman’s abnormal smear that have not previously been recorded within TOMBOLA. These might include colposcopy examinations occurring within the NHS and any related biopsies, treatment and histology, any episodes of hospitalisation and additional smears. This exercise will yield
information on CIN2/3, and also information that will feed into the analysis of other outcomes (see later). In addition, women are asked at their exit examination if they have undergone any additional procedures outwith the local NHS setting, for example a smear or examination in a private hospital. Such procedures are very rare among women participating in TOMBOLA.

For women who have left the trial before their exit appointment, information about any non-TOMBOLA appointments is obtained from hospital records and pathology databases.

The point prevalence of CIN2/3 detected solely at the exit examination will also be considered as an endpoint. CIN2/3 that is detected at the exit examination is either a result of failure to detect CIN2/3 earlier or treat detected CIN2/3 adequately or newly incident disease which has developed during the interval since the last examination or treatment. Distinction between these origins of CIN2/3 can only be made arbitrarily.

CIN3 or worse will also be considered as an endpoint. In addition, any cases of disease more severe than CIN3 will be documented.

4.1.2. HPV status

As described earlier, HPV status is being assessed at recruitment into the trial, and also at the end of the three-year follow-up. In addition to classifying women as high-risk HPV positive or high-risk HPV negative using the GP5+/6+
consensus primers (described earlier), additional tests are being conducted on the HPV samples including identifying specific HPV types (including types 16 and 18) and estimating viral load (ie how much virus is present).

4.1.3. Complications

Immediate complications occurring in women during a colposcopy or treatment appointment are recorded at that time by clinic staff. These include haemorrhage, fainting and nausea. Short- and longer-term complications of the colposcopy and treatment procedures are being elicited from women using questionnaires sent out 6 weeks after the last appointment (which may be a colposcopy or a treatment appointment) and 4 months after the first colposcopy appointment. These questionnaires seek information on bleeding, pain or discomfort, infection, disrup
tion to menstrual cycle and any change the woman has had to make to her usual method of contraception, such as removal of an IUD coil in order to have a LLETZ. A similar questionnaire is sent to women in the cytological surveillance arm 6 weeks after their first smear in primary care.

4.1.4. Default

There are a number of points at which women can default during follow-up. Default is defined as non-attendance for an intervention a woman is scheduled to have, including 6-monthly smears, colposcopy appointments, treatment appointments, and follow-up after colposcopy. Attendance at appointments or for smears is recorded, and from this information, default can be assessed.

4.2. Psychosocial outcomes

There are three strands of psychosocial work within TOMBOLA.

First, TOMBOLA is comparing the medium term psychosocial effects of cytological surveillance versus colposcopy by assessing the cumulative proportion of clinically significant anxiety and/or depression over the three-year follow-up. This is determined by the Hospital Anxiety and Depression Scale (HADS) [17]. A process-specific instrument (Process Outcome Specific Measure; POSM) has been developed to capture information on more subtle, but potentially important, psychosocial issues including cancer-related worries, effects on sexual function, concerns about fertility and anticipated future participation in cervical screening. The development of this questionnaire has been described in detail elsewhere [18,19]. The HADS and POSM, together with the EQ-5D [20] (see section on Economic outcomes) are combined into a single questionnaire booklet that is administered at recruitment and by post at four time-points during follow-up — 12, 18, 24 and 30 months after recruitment. The timings of these administrations were chosen to avoid picking up “spikes” of anxiety or short-term distress associated with colposcopy or cytology procedures scheduled to happen in the three-year follow-up period.

The Multi-dimensional Health Locus of Control Scale (MHLCS) [21] is included in the recruitment questionnaire booklet. This instrument assesses three dimensions people believe exert control over their health — “powerful others externality”, “chance externality” and “internality”. The MHLCS may explain differences in the main psychosocial outcomes, and will be considered a covariate in the analyses. It will also be considered as an explanatory variable in relation to other outcomes, such as default.

Second, TOMBOLA is evaluating the short-term psychosocial effects of immediate treatment versus biopsy and selective recall. Questionnaires are administered at colposcopy and treatment appointments and include the HADS, six-item version of the state component of the Spielberger State-Trait Anxiety Inventory (STAI) modified by Marteau and Bekker [22], a 12-item Eysenck Personality Inventory (EPI) [23] and the EQ-5D [20]. The STAI measures anticipatory anxiety in relation to the planned colposcopy or treatment. The EPI measures the personality traits of extroversion, introversion and neuroticism; personality traits may be explanatory variables for other psychosocial outcomes.

A further psychosocial assessment is made, by post, 6 weeks after the colposcopy. Two psychosocial instruments are included in the booklet: the HADS and the Impact of Events Scale (IES) [24]. The IES includes two sub-scales (avoidance and intrusion) and measures the extent to which an event (ie in this case having a colposcopy) affects people’s functioning on a day-to-day basis. In addition to the two psychosocial instruments, the questionnaire booklet also contains the EQ-5D and the questions on short-term complications of management described earlier.

Third, focus groups were convened to obtain detailed information regarding the views of women about the management of their abnormal cervical smear and the acceptability of their management. These were conducted in each of the trial areas in 2003 and included women who had been recruited into TOMBOLA around 12 months previously.
Two of the groups included women who had been randomised to the colposcopy arm of the trial and one group included women who had been randomised to the cytological surveillance arm of the trial.

4.3. Economic outcomes

In order to assess the cost-effectiveness of the alternative management policies, NHS resource use in primary and secondary care is being recorded. This includes the resources associated with taking and reporting cytological smears, of undertaking a colposcopy and any related treatment, and of histological diagnosis. Resource use within TOMBOLA clinics and in NHS clinics will be included. Unit costs, derived from both national and local sources, will be assigned to each resource item.

Resource use associated with HPV testing, including collection of samples and the testing itself, is being collected, and where not available, will be estimated, and will be included in the assessment of the cost-effectiveness of using HPV testing in triage.

The costs that are borne by women and their families in relation to the interventions are also being assessed [19]. These include travel costs, travel time, time-off work and costs relating to alternative care for children or other dependants. In the initial colposcopy arm, women are sent a questionnaire with their appointment letter, asking them to complete the questionnaire in relation to the colposcopy appointment. Women in the biopsy and selective treatment arm who have to return for treatment are sent a similar questionnaire with their appointment letter and asked to complete it in relation to the treatment appointment. In the cytological surveillance arm, women are sent a questionnaire when their first smear in primary care was due and asked to complete it in relation to attending for the smear. These data will be used to derive an average cost per woman for each management alternative.

Health-related quality-of-life is being assessed at various time-points during the trial (described above) using the EQ-5D [20]. This is a generic instrument, and will enable comparison of the effect on health-related quality-of-life of TOMBOLA interventions with interventions for the treatment of other conditions and with the general population [19].

4.4. Other information collected

At recruitment, women were asked to complete a socio-demographic questionnaire. This collected information on ethnic group, marital status, education since leaving school, employment and occupation, contraception use, use of hormonal drugs, pregnancies and childbirth, use of pain-killers, smoking habits and physical activity. This information will be used to describe the characteristics of participants, to check the balance of randomisation, and included, as covariates, in other trial analyses.

5. Quality assurance

The cytopathology and histopathology laboratories in the three trial centres participate in national quality assurance schemes. In addition, quality assurance exercises are being undertaken to quantify levels of consistency in histological and cytological diagnosis within and between the trial centres.

A slide circulation has been undertaken to assess consistency in cytological grading. Slides from different smear gradings were circulated between cytopathologists in each of the trial centres. The results of this inter-observer comparison will be reported elsewhere.

In regard to histological diagnosis, a panel of 8 histopathologists working in laboratories where TOMBOLA histology samples are read or in other UK laboratories participated in a consensus workshop in 2002. Twenty-one histology slides covering the spectrum of cervical pre-malignancy and carcinoma were circulated and read by participating histopathologists. For 16 slides, there was complete agreement, or agreement by all but one pathologist, on the CIN grade (high-grade — CIN2 or more; low-grade — CIN1 or less). A central review process is currently being set up where a random selection of cases from TOMBOLA will be reviewed by one of two pathologists. If this agrees with the original diagnosis, no further review will be undertaken. However if it does not agree, a third reviewer will make a diagnosis and the majority decision recorded. The results of this review will be reported elsewhere.

All of the colposcopists undertaking colposcopy examinations within TOMBOLA are accredited by the UK national body (British Society for Colposcopy and Cervical Pathology) following training. Agreement in colposcopic diagnosis among TOMBOLA colposcopists is also being assessed. A series of 2–4 colposcopic images of the application of acetic acid to the cervix have been compiled from 129 women from one of the trial centres. The images were reviewed
by six trial colposcopists to judge whether an area of abnormality is present; levels of consistency are being assessed, and the results will be reported elsewhere.

As regards the HPV analyses, known positive controls from a commercial source are included in each batch of assays. The HPV laboratory has exchanged samples with a European laboratory to confirm positivity and HPV typing. In addition, the laboratory has recently registered with an International Quality Assurance Panel and will participate in their quality assurance scheme.

6. Statistical power

Power calculations were undertaken when the trial was being planned. These were revised in light of the changes to eligibility criteria. In the comparison of cytological surveillance versus initial colposcopy, TOMBOLA has:

- 80% power to detect a relative risk of 1.15 in cumulative incidence of CIN2/3, and 95% power to detect a relative risk of 1.2 (assuming an overall cumulative incidence of CIN2/3 of 15% and \( \alpha = 0.05 \) for a two-sided test).
- 80% power to detect a difference of 6% in the cumulative proportions of clinically significant anxiety and/or depression, and 90% power to detect a difference of 7% (assuming an overall cumulative proportion of clinically significant anxiety and/or depression of 30% and \( \alpha = 0.05 \) for a two-sided test); and
- 85% power to detect a relative risk of 1.3 in cumulative incidence of CIN2/3 between the management arms in women who are HPV positive, and 97% power to detect a relative risk of 1.4 (assuming an overall cumulative incidence of CIN2/3 of 15%, 33% of women HPV positive and \( \alpha = 0.05 \) for a two-sided test). The power to detect, as statistically significant, an effect in HPV positive women provides an indication of the power to detect an interaction between HPV status and management policy.

In the comparison of immediate LLETZ versus biopsy and selective treatment, the trial has 86% power to detect a relative risk of 1.25 in the cumulative incidence of CIN2/3, and 95% power to detect a relative risk of 1.3 (assuming an overall cumulative incidence of CIN2/3 of 15%, that 5% of women decline randomisation R2 and \( \alpha = 0.05 \) for a two-sided test).

7. Analysis

Analysis will be conducted on the basis of intention-to-treat. Comparison will be made between (i) the cytological surveillance and initial colposcopy arms and (ii) the immediate LLETZ and biopsy and selective recall arms. In the evaluation of cytological surveillance versus initial colposcopy, the following outcomes will be compared: cumulative incidence of CIN2/3 (ie CIN2/3 or more severe disease), cumulative proportion of clinically significant anxiety and/or depression, and default from management policy. Sensitivity analyses will be undertaken on the basis of severity of index smear and trial centre. Additional analyses will focus on CIN3 or worse. Secondary outcomes, such as those assessed using the POSM and point prevalence of CIN2/3 at the exit examination will also be considered. In the evaluation of immediate LLETZ versus biopsy and selective recall, the cumulative incidence of CIN2/3 and default from treatment policy will be compared. Psychosocial effects of the treatment policies will be compared using women’s responses to the Impact of Events Scale and the HADS. The frequency of complications related to colposcopy, immediate LLETZ and biopsy and selective recall will be assessed, and compared with the incidence of complications in cytological surveillance. The economic analysis will be concerned with the relative efficiency of alternative policies for managing and treating women with low-grade cervical abnormalities. This will involve the synthesis of cost and outcome data in order to derive incremental cost-effectiveness ratios. The uncertainty in the cost-effectiveness estimates will be evaluated using cost-effectiveness acceptability curves [25]. Further details of the economic analysis can be found in Philips et al. [19].

To evaluate the potential contribution of information on HPV status to management and treatment, possible interaction between the alternative management and treatment policies and HPV status will be investigated in relation to the CIN2/3 outcomes. The frequency of CIN2/3 or worse will be compared between the arms of the trial in (i) the group of women who are high-risk HPV-positive on entry to the trial and (ii) the group of women who are high-risk HPV-negative on entry to the trial.

In addition, if we show that HPV testing could be clinically effective in the triage of women with low-grade abnormalities, we propose to investigate the potential economic consequences associated with the addition of this
testing to the various management policies. The marginal cost-effectiveness and cost utility of the addition of HPV testing to the current screening protocol will be estimated using mathematical modelling techniques.

Mathematical modelling techniques will also be employed to predict long-term NHS resource use and cost, cases of cervical cancer and life-year estimates, and long-term cost-effectiveness.

8. Trial organisation

The trial is managed according to MRC guidelines [26]. A Trial Steering Committee provides overall supervision for the trial. The Committee includes independent members and a chairperson who are not otherwise involved in the trial. A Data Monitoring and Ethics Committee reviews unblinded data from the trial, and has opportunity to make recommendations to the Steering Committee on any ethical or safety issues relating to the trial. Members of this committee are independent both from the trial and the Steering Committee. The Trial Steering and Data Monitoring and Ethics Committees meet annually.

The trial grant-holders are from multi-disciplinary backgrounds, including epidemiology, medical statistics, gynaecology, health economics, health services research, cytology, pathology and HPV research. The Trial Management Group, incorporating trial grant holders and study staff meets around once a year. The Trial Executive Group meet quarterly by telephone conference to manage the trial. This group includes the chair of the Management Group, representatives from each of the centres and the Trial Manager. Specialist interest groups, made up of grant holders and study staff, meet to take forward aspects of the research; for example the Recruitment Strategy Group considered strategies to boost recruitment into the trial, the Gynaecology Group have driven the clinical implementation of the trial and the Health Services Research Group developed the psychosocial and health economic aspects of the trial.

Study staff (including local co-ordinator, clerical assistant, trial colposcopist and research nurses) are employed in each centre to undertake recruitment and follow-up of women. In addition, specialist researchers are employed to undertake the work on health economic and psychosocial aspects and specialist technicians undertake the HPV testing. A data analyst undertakes data management activities and ensures data quality and a statistician will be employed to undertake final analyses of trial data. A Trial Manager oversees the day-to-day running of the trial. Programming support has been in place to develop and maintain the highly complex trial databases. Cytological and histological diagnosis are undertaken by the routine pathology services in each of the centres.

9. Current status

Recruitment to the trial closed on 31 January 2003. Almost four and a half thousand women were randomised at R1 (ie to either colposcopy or cytological surveillance). Thirty percent of these were recruited during the first recruitment phase and the remainder during the second recruitment phase. Around one-third of women randomised had a mild index smear. The recruitment rate was 52%. Of those randomised to colposcopy, just over 2000 women were randomised at R2 (ie to either immediate treatment or biopsy and selective recall). Just over half of the women attending for colposcopy had an abnormal transformation zone, and therefore underwent their R2 procedure. Only 26 women who consented to randomisation R1 and were eligible for randomisation R2 did not consent to this second randomisation. Follow-up was completed in December 2005. Almost 70% of women who have been invited to attend their exit appointment have attended. Information from medical records in relation to any colposcopy appointments the woman has attended during her follow-up (and any related histology) has been recorded for all trial participants.

10. Discussion

When the joint commissioning brief for a randomised-controlled trial of alternative referral and management policies for women with low-grade smears was issued, the original design proposed by the MRC included an arm where HPV status would be disclosed and women who were HPV positive would be referred for immediate treatment. In designing TOMBOLA, the investigators felt that this would be scientifically sub-optimal for a number of reasons. First, such a design would result in confounding between management policy and HPV status, and it would be impossible to disentangle the effects of management from the effects of HPV. Second, there was insufficient scientific evidence to justify management based on an HPV result. Third, it was not possible to estimate with confidence the
proportion of women who would be high-risk HPV positive at enrolment. For these reasons, TOMBOLA was designed with two principal arms, immediate colposcopy and cytological surveillance, and allocation to these was not based on HPV result, in order that interactions between HPV test result and the alternative management and treatment policies could be tested in relation to cumulative incidence of CIN2/3. This approach will enable an unbiased evaluation of the role of HPV in triage. Further justification for the two-arm approach adopted in TOMBOLA was provided by the ASCUS-LSIL Triage Study (ALTS) [27]. This trial was designed with 3 arms, immediate colposcopy, “conservative management” (repeat cytology) and “triate” based on the result of an HPV test in combination with cytological smear. In the triage arm, women were referred to colposcopy if their enrolment HPV test was positive or “missing”, if they had an enrolment smear of high-grade squamous intraepithelial lesions (HSIL), or a smear showing HSIL during follow-up. However, the triage arm closed to women with low-grade squamous intraepithelial lesions (LSIL, similar to the UK definition of mild dyskaryosis) more than a year early because of the high rates of HPV positivity in these women.

At the outset of TOMBOLA we were not able to estimate with confidence the proportion of women who would be high-risk HPV positive in our population. At that time few studies had been published on the prevalence of HPV infection and those that had been published tended to include highly selected populations from other countries, for example students or young women in Sweden [28] or women attending a gynaecology clinic in the USA [29]. It was not clear to what extent rates of HPV infection in these individuals would apply to women aged 20–59 in the UK. In order to ensure that there was a balance in the proportion of women who were high-risk HPV positive between the trial arms, both randomisations were stratified by HPV result. Using stratification to achieve a balance within the R2 comparison (immediate treatment versus biopsy and selective treatment) was particularly important because of the smaller number of women involved.

The design of TOMBOLA permits an unbiased evaluation of the role of HPV testing in triage of women with low-grade smears. The investigators were also concerned about the impact on the psychosocial evaluations in TOMBOLA if women were aware of the HPV testing element of the study. The logical extension of the design was to conceal the HPV result from both clinicians and from subjects. In practice, it has not been problematic to conceal the HPV result from the colposcopists or from the cytology screeners, nor has it been an issue for participants. The few women (<1%) who asked about the additional testing were told that it was for HPV. At present, it is not clear what might be the psychological or psychosocial impact of unblinded testing for HPV. Recent research in the UK has suggested that women with a low-grade smear who test positive for HPV are more anxious and distressed (in the short-term) than women who test negative for HPV or who are not tested [30], but that after 6 months there is no difference [31]. However, knowledge of the value of HPV in triage (which is one of the specific objectives of TOMBOLA) would be likely to change the information given to women in the future, and this in turn would be expected to alter the psychological impact. Moreover, it would be expected that randomisation will have distributed the women who asked about the testing equally between the different arms of the study so any psychological or psychosocial differences will have a minimal impact on the comparison between trial arms.

At the time TOMBOLA was designed, an initial Zelen randomisation [32], (R0) was intended to operate during the first year of recruitment to precede randomisation R1. It was planned that eligible women would be randomised to (i) be invited to participate in the trial or (ii) act as a “current local practice” group who would be managed according to local practice in that area but asked to complete questionnaires on psychosocial well-being. The purpose of this was to consider the possibility that participation in a trial involving recruitment through study centres may in itself influence psychosocial well-being and mask differences in psychosocial well-being in women managed by cytological surveillance compared with those undergoing initial colposcopy. In addition, it would assess the effect of the organisation of the trial and the information given on anxiety levels and psychosocial well-being. The inclusion of this randomisation was to aid in generalising the findings of the trial to the cervical screening programmes. However, after the first meeting of the Trial Steering Committee, during the implementation phase of TOMBOLA, this initial randomisation was dropped. The reasons for this were three-fold. First, it was agreed that the logistical difficulties in implementing R0 far outweighed the potential benefit this randomisation would bring to the project. Second, the rationale for including such a randomisation was scientifically unconvincing. Third, the removal of this randomisation enhanced the recruitment potential of the trial as all eligible women would be invited to participate in TOMBOLA. This was fortunate, given the subsequent challenges with recruitment (ie lower numbers of eligible women than had originally been anticipated).

Developing and running trials in a context of rapid changes in clinical opinion and practice (such as cervical screening) present challenges, especially when the trial is of relatively long duration. A changing climate of opinion can
force investigators to change the research question being addressed, or can even make the research question redundant. In TOMBOLA the changing climate of opinion enabled us to broaden the eligibility criteria, and address the same research question in a “new” group of women (ie those with a single borderline smear). Had we not done this, we may have found, at the end of the trial, that the debate had overtaken TOMBOLA, and the results would have been redundant. There has also been commercial pressure (from a company marketing HPV testing kits) to introduce HPV testing into the UK screening programmes [33,34]. This is despite a continued lack of robust evidence on the value of HPV testing in the triage of women with low-grade abnormalities. To date HPV testing has not been introduced into the UK screening programmes, but a number of pilot projects have been undertaken to evaluate issues associated with its possible introduction; the results of these are awaited [12]. However, these pilot projects will not provide evidence on the effectiveness of HPV testing in triage, which will be available from TOMBOLA. There are also ongoing developments in cytology. In 2001 liquid-based cytology (LBC) was piloted in three health regions in Scotland, including Grampian and Tayside, where LBC was used in selected GP practices. LBC was introduced across Scotland, in place of traditional cytology during 2003/4 [35]; this has been achieved. In England, LBC will also replace traditional cytology, with the new technology being rolled-out over the coming five years [36]. The residual from LBC can be used for HPV testing. In the pilot implementations of LBC in England and Scotland, there were reductions in the proportions of smears reported as inadequate, but no overall effects on low-grade abnormal smears as a proportion of adequate smears [37,38]. Although unlikely, it is possible that the characteristics of women with liquid-based smears classified as low-grade may differ from women with traditional cervical cytological smears classified as low-grade. This will be evaluated within TOMBOLA and, if necessary, the potential impact on generalisability of the trial results will be assessed. Following the British Society for Clinical Cytology Terminology 2002 Conference, it has been suggested that the way in which cytological abnormalities are graded and classified might be modified, but this has been open to debate [39]. For example, there is concern among colposcopists that BNA smears with koilocytes will be classified as “mild”. With the recent NHS CSP guidelines for England recommending referral to colposcopy on an initial mild smear [3], such classification could result in a large increase in service demand for colposcopy. However, provided that the borders between normal and “low-grade” and “low-grade” and moderate do not change, the generalisability of the results from TOMBOLA should not be affected.

Throughout the design and implementation of TOMBOLA, we have been concerned about possible over-treatment of women. This has guided many of the decisions taken. The eligibility criteria in the first phase of recruitment were designed to ensure that eligible women had persistent cytological low-grade changes. One of the major factors leading to the change in eligibility criteria to include women who had a single smear showing BNA was in response to an increasingly interventionist approach to management of women with low-grade abnormalities, with increasing proportions being referred to colposcopy after a single low-grade smear. Since TOMBOLA had been funded, the debate on management of women with low-grade abnormal smears had moved on to the extent that the question of what to do about women with a single BNA smear was being debated. It was considered that TOMBOLA might be the last (and very timely) opportunity to determine the most effective and efficient management for these women, and potentially to conclude that such interventionist management is unnecessary for the majority. The trial includes a policy of cytological surveillance, with referral to colposcopy only after a smear showing moderate dyskaryosis or worse. Previous studies had shown this policy to be safe in a research environment [40], although NHS CSP guidelines at the time suggested referral to colposcopy after three BNA or two mild smears [4].

Another reason why a change in eligibility criteria was implemented was that the number of eligible women was much lower than anticipated. When the trial was designed, it was difficult to estimate the numbers of eligible women because the available screening data was based on the numbers of smears taken, and not on numbers of women. While smear-based information is essential for workload estimates in cytology laboratories, it is not useful in terms of assessing the public health impact of screening (for example, what proportion of women have an abnormal smear each year) or in terms of the workload at GP surgeries or colposcopy units. We would therefore advocate that screening programmes produce both women-based and smear-based data. As women-based information was not available, the number of eligible women was estimated from smear-based data in consultation with colleagues in cytology and gynaecology. In the initial implementation phase, database programming for the bespoke TOMBOLA database enabled us to produce, for the first time, women-based smear information [14]. This suggested that the overall number of women having low-grade abnormal smears was smaller than anticipated because many of the women were having a series of low-grade smears. Based on this information, we were able to make a more accurate estimate of the numbers of eligible women.
TOMBOLA is a pragmatic trial, nested within the cervical screening programmes in England and Scotland. This presented a number of challenges for the study team, primarily in ensuring that the trial could run alongside the screening programmes without encumbering them and that the management of women would not be compromised by any problems arising from the interface between the trial and the screening programmes. Therefore, considerable liaison was needed between trial team and local cytology departments, who manage the routine screening programme. A time-point was identified between smear results being read and the results being sent to women and/or GPs where the study team could intervene in order to identify eligible women, and offer the opportunity to become involved in the trial before routine cytology protocols were initiated. An elaborate custom-written database was required to interface with the cytology systems, which were different in each area. The database was complex and time-consuming to develop, and therefore expensive. ‘Fail-safe’ systems have also been needed at several points to ensure that women who did not want to take part, or decide to leave the trial, are captured back in the routine screening programme and referred onwards for appropriate care. Women participating in the trial also had to be identified on the local cytology databases to ensure they were managed according to the TOMBOLA protocol and not local cytology protocols.

An additional complication of this pragmatic trial is the heterogeneity that exists both within, and between, centres. This heterogeneity is present in diagnosis and interpretation of cytology, histology and colposcopic impression, and in management policies. Cytopathology and histopathology samples taken from TOMBOLA participants are read in local pathology laboratories as part of the routine pathology service, and these laboratories participate in national and local quality assurance schemes [41,42]. Colposcopists working in TOMBOLA are all accredited by the British Society for Colposcopy and Cervical Pathology. In addition, TOMBOLA quality assurance schemes have been implemented to document levels of consistency in interpretation of cytology and histology slides, and of colposcopic impression. Much of the heterogeneity relating to management policy is removed by the imposition of the trial structure and the randomisation of women to one policy or another. During the run-in phase of the trial, additional areas of heterogeneity were identified, and in some cases additional trial protocols were implemented to reduce this heterogeneity. For example, while follow-up after colposcopy was originally defined as “according to local practice”, it became apparent that this differed markedly between centres (either colposcopy or repeat smears). To combat this, a standard protocol for follow-up after colposcopy was agreed across centres (repeat smears for the majority of women). Other areas of heterogeneity in management were accepted and no attempts made to standardise policy between centres. For example, in one centre women with IUD coils were not usually treated with LLETZ, the cervical transformation zone being destroyed using the Semm “Cold” Coagulator instead. Heterogeneity reduces study power and in some cases can mean that it is unclear what management/treatment policies are actually being evaluated. While it was important to standardise practice to some extent between centres, it was also important to do this without creating an artificial “trial” situation, which could compromise the pragmatic nature of the trial and the generalisability of trial results to the very heterogeneous environment of the cervical screening programmes.

There are two randomisations within TOMBOLA — R1 to cytological surveillance or colposcopy, and within the colposcopy arm, R2 to biopsy and selective recall or immediate treatment, and because of this a two-stage approach to consent was adopted. In the design phase, consideration was given to seeking consent for both R1 and R2 at recruitment. It would also have been possible to adopt an approach where women were randomised to either (i) cytological surveillance, (ii) colposcopy and immediate treatment and (iii) colposcopy and biopsy and selective recall. However, both of these options would have involved decision-making about treatment options following colposcopy (immediate LLETZ versus biopsy and selective recall) for women who would not be randomised to colposcopy. It was felt that adopting a two-stage approach to consent gave women a full opportunity to reflect and discuss their decision regarding participation in each component of the trial, which is in line with current views on “fully informed” consent.

In TOMBOLA, recruitment of women was undertaken in hospital-based recruitment clinics. During the design phase of the trial, consideration was given to recruitment of women in their GP surgery. If women had been recruited this way, cluster randomisation, by GP practice, would have been employed. However, there was concern about the fact that cluster randomisation is likely to be less efficient than randomisation of individual women, because individuals contribute less information [43]. The overall sample size required to achieve comparable statistical power would be greater, and the magnitude by which the sample size would have had to be increased was uncertain because of lack of information on intra-practice correlation in the characteristics of women patients [44]. In addition the logistics of obtaining an HPV sample at recruitment through a network of GP surgeries would have been complex. A potential drawback to hospital-based recruitment is the potential negative impact it may have on participation. On balance, it was decided to proceed with recruitment through clinics and individual randomisation, and with this strategy we achieved a participation rate of 52%.
In planning TOMBOLA it was difficult to estimate what the participation rate might be because of a lack of population-based studies. A non-randomised comparison of follow-up for women with mild dyskaryosis in the UK had reported a participation rate of 83% [45]. In that study, women were recruited from those attending a colposcopy clinic and so the participation rate was likely to be higher than could be achieved from a screening population. Other UK studies comparing alternative follow-up policies had not reported participation rates [40,46,47] (some of these were published before the CONSORT statement [48]). The participation rate in TOMBOLA remained static around 52%, despite strenuous efforts to increase it. Trials of follow-up published more recently have demonstrated participation rates of 75% [27] and 42% [49]. The ALTS trial, which achieved a participation rate of 75%, was conducted in the US, where women with a low-grade cervical smear may expect to attend a hospital appointment with a gynaecologist (which would not necessarily be expected in the UK); and this may in part explain the higher recruitment rate into the ALTS trial. Women were only eligible for the ALTS trial if they were likely to participate for the full duration of the trial [27]. In addition, “modest financial incentives” were used to help retain women in the ALTS trial [50]. No such incentives were offered for recruitment into, or attendance for colposcopy, treatment or smears in TOMBOLA.

As part of TOMBOLA we have undertaken research to identify factors associated with participation and non-participation. This involved looking at characteristics (including age, deprivation, distance from home to hospital clinic) of participants compared to non-participants [51]. Preliminary analysis showed that participation rates were lower in (i) younger women (46%) compared to older women (63%) and (ii) women resident in more deprived areas (45%) compared to less deprived areas (55%). Distance to clinic was not associated with participation. We also undertook questionnaire-based research in women who had either agreed or declined to participate in TOMBOLA [52]. The most common reasons women gave for not taking part were that they would prefer follow-up from their own GP, clinic appointment times were inconvenient, they had problems getting time off work, the cost of travelling was a concern, and child-care was an issue. Of participants, over 70% cited an altruistic reason as the most important reason for participation (for example they wanted to make a contribution to the screening programme or help in medical research), and a third of women thought that participation would result in better care. Full results will be published elsewhere.

Whilst the goal of the cervical screening programmes is the prevention of cervical cancer, cancer is not the primary endpoint of the trial. This is because diagnosis of cancer can be considered a screening failure. It is therefore important to have a surrogate endpoint. Cervical screening works by detecting and treating CIN. While CIN3 is considered to be a precursor lesion for cancer, in that if left untreated it could progress to cervical cancer, the boundary between CIN2 and CIN3 is not entirely clear and, in fact, there is considerable inter-observer variation in grading CIN [53,54]. It is for this reason that CIN2/3 (rather than just CIN3) has been chosen as the primary clinical endpoint of TOMBOLA. This endpoint has been used in previous studies in cervical screening as a surrogate for invasive cancer [27,40,45,46]. Secondary analyses in TOMBOLA will relate to CIN3 alone. Mathematical modelling techniques will be employed to extrapolate information about CIN2/3 in the trial population to cancer incidence and mortality. In the longer term, we plan to link the trial cohort with population-based cancer registries to identify cases of cancer.

There are challenges in following-up participants for a three-year period. As all smears taken within each trial area are read centrally, and reported to the TOMBOLA team, women’s address changes can be tracked within the local area. Women who move outwith the local area are more difficult to locate, but this has been achieved for the majority and a limited set of outcome data will be sought for these women. We have used newsletters for participants to help keep in contact. In addition, many participants will have received questionnaires during their follow-up period, and it is hoped that this will make them feel part of the trial. Strenuous efforts are made to encourage women to attend their exit appointment. Two appointments are offered to each woman, with an opportunity for them to contact the centre and re-arrange the appointment to a more convenient time. Women’s addresses are checked prior to appointments being made, and again if a woman does not attend for an appointment. Research is also underway to attempt to find out why women do not attend for their exit appointment, and whether there was anything else that could have been done to encourage them to attend.

TOMBOLA will considerably enhance the current knowledge-base on the psychosocial effects of having an abnormal smear and its subsequent management; the complications of colposcopy and treatment (from the woman’s point of view); the costs of management and treatment to the NHS, and, provide for the first time, the costs borne by women. There has been limited previous research in these areas. The studies of Bell et al. [47] and Jones et al. [55] investigated psychosocial effects of the management of an abnormal smear in the UK, but both studies had methodological limitations. In the only large UK study of self-reported complications which included 1696 women
undergoing LLETZ, vaginal discharge and bleeding were reported by the majority of women [56]. Costs of management and treatment borne by the NHS were estimated in the studies of Jones and Jones [57] and Flannelly et al. [58] and in work by Waugh et al. [59]. In part due to the limited previous research in these areas, but also because of the pragmatic nature of TOMBOLA and the different pathways women follow through the trial, it has been challenging to develop these novel aspects of the trial. In particular, developing appropriate instruments and identifying appropriate time-points for administration has been difficult.

The primary outcome for the psychosocial component of TOMBOLA is the HADS [17]. However, we could not identify an appropriate “off-the-shelf” instrument to measure the other, less extreme but potentially important, consequences of having an abnormal smear and its subsequent management, including, for example health and cancer-related worries, adverse effects on sexual activity and relationships, fertility concerns and future participation in cervical screening. Such an instrument was developed for TOMBOLA, and the development process is described elsewhere [18]. It was designed to assess the impact of having an abnormal smear, and also to assess the impact of the management of this. Whilst immediate complications of a procedure are documented at the TOMBOLA clinic, an instrument was required to measure medium and longer-term complications of colposcopy and treatment. Again, in order to measure these complications, instruments were developed, piloted and implemented. It is difficult to assess complications as the interpretation of what is “normal” is likely to differ between individuals, however this is why it is important to ask women directly about any after-effects they experienced. In addition, there may be after-effects that women consider to be important and to have an adverse effect on their lives, which may not be considered clinically to be a complication. Questionnaires designed to assess the time, and costs to women (including travel, childcare, time off work) were developed from existing time and travel cost questionnaires [19].

Careful consideration was given to the timing of administration of the instruments. In part, this was to avoid overloading women with questionnaires at a specific time-point during their follow-up. The timings of some psychosocial questionnaires were also chosen to avoid ascertaining short-term distress associated with colposcopy or cytology procedures scheduled to happen during the 3-year follow-up period. Other questionnaires were timed to be administered at a specific time after a procedure.

The TOMBOLA trial provides a valuable platform for additional research. Research using the samples taken for HPV testing is just one aspect of the additional research that is either underway, or planned. Primary HPV testing within TOMBOLA involves PCR-EIA methodology using GP5+/6+ consensus primers. This methodology has been used in other large studies, for example PROBASCAN (Population Based Screening Study Amsterdam) [60] and has been shown to have reasonable agreement with a commercially available testing kit (Hybrid Capture II [61]. The primary methodology uses only part of the sample collected for HPV testing, leaving residual for additional testing. Real-time PCR is being undertaken on the residual, using consensus primers, and probes for specific strains of HPV [62,63]. This will generate considerable information about the role of type-specific infection and the clinical significance of viral load. Studies involving mRNA gene expression are also underway. Analyses of socio-demographic and lifestyle factors associated with HPV infection are being undertaken. TOMBOLA will also provide a platform for work on host and viral genetic factors, and projects in this area are being developed.

The results of TOMBOLA are intended to inform the way in which women with low-grade abnormal smears are managed in the future. Currently the TOMBOLA team are considering strategies to ensure widespread dissemination of the trial results, and also how to ensure that the results are considered (together with those of other studies of women with low-grade abnormalities and HPV testing [11,12]) in the development of new management guidelines. For TOMBOLA, it is hoped to achieve this by close liaison with the cervical screening programmes.

The ALTS trial in the USA has recently reported on three alternative methods of managing women with low-grade and equivocal cytologic diagnoses — (i) immediate colposcopy, (ii) HPV triage based on HPV testing in combination with cytology and (iii) repeat cytology [50,64]. However it is unclear how generalisable the evidence is to the UK setting. The populations who participate in screening and the recommended screening protocols are very different between the UK and the USA. In addition, cytology classification and the definition of incident and persistent abnormalities are different between the two countries. A number of other studies within the UK are assessing the value of HPV testing. These include the HART study (HPV in Addition to Routine Testing) [11], ARTISTIC (A Randomised Trial In Screening To Improve Cytology) [13] and the NHS HPV pilots [12]. The HART study assessed the detection rate and positive predictive values of HPV testing with cytology, and considered management strategies for women aged 30–60 who tested HPV positive, and results have recently been reported [11]. The ARTISTIC trial is considering the implications of introducing HPV testing into primary cervical screening, and is not due to report until 2008 [13]. A number of HPV pilot projects have been
undertaken within the NHS CSP in England [12,65]. These were designed to evaluate a number of issues associated with introducing HPV testing into the cervical screening programme as triage for smears showing borderline nuclear change and mild dyskaryosis; but with limited follow-up. The results from TOMBOLA will complement the information provided by these other studies.

TOMBOLA addresses an important question for the UK cervical screening programmes and for other populations in which screening is undertaken: what is the best management for women with low-grade abnormal smears. The design of TOMBOLA enables three aspects of this question to be addressed in terms of both effectiveness and cost-effectiveness: comparison of cytological surveillance and initial colposcopy; within colposcopy, comparison of biopsy and selective recall for treatment and immediate treatment; and evaluation of the potential role of HPV testing in management. Final results will be available in 2006/2007.

Appendix A. TOMBOLA Group

TOMBOLA Grant-holders

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References


