Autologous Bone Marrow Derived Mononuclear Cell Therapy in Muscular Dystrophy: A Review

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Abstract

Muscular dystrophies are a heterogeneous group of genetic disorders characterized by progressive muscle degeneration of variable distribution and severity. There is no known definitive treatment.Cell therapy holds promise as an interim treatment approach that can improve the quality of life of these patients.We reviewedthe published literature,assessing the safety and efficacy of autologous bone marrow mononuclear cell therapy in muscular dystrophy. An online search revealed 13 studies including 3 case series and 10 case reports with a total of 257 patients. All the preliminary studies met the prespecified criteria for quality and were of fair to good quality.All the studies administered cell therapy using intrathecal and intramuscular routes. There were no procedure related major adverse events in any of the studies. Manual muscle test,Functional Independence Measure and Brooke and Vignos scales were the most commonly used outcome measures in the studies. All the studies demonstrated improvement on functional scales and 12 studies demonstrated improvement in muscle strength post intervention. The studies showed that cell therapy using autologous bone marrow mononuclear cellsis safe and effective inslowing down disease progression, and maybe an effective interim treatment option.

Keywords: Muscular dystrophy, autologous, bone marrow, mononuclear cells, cell therapy

Introduction

Muscular dystrophies are a heterogenous group of genetic disorders characterized by progressive muscle degeneration of variable distribution and severity. They are inherited as X-linked, autosomal recessive, or dominant disease. There are seven major forms of (MD) including Duchenne muscular dystrophy (DMD); Becker muscular dystrophy (BMD) ; Emery-Dreifuss; Fascioscapulohumeral; Oculopharyngeal; Limb Girdle muscular dystrophy (LGMD) and Congenital muscular dystrophy (CMD) [1]. The genetic basis of all types of MD involves defects in different component proteins of an elaborate dystrophin glycoprotein complex (DGC) that bridges the inner cytoskeleton and the extracellular matrix of a muscle fiber [2]. The resulting disruption of the complex, causes destabilization of the muscle membrane, increased muscle fragility and wasting. Repeated cycles of injury and repair follow which exhaust the resident stem cell pool and muscle is eventually replaced by fibrofatty tissue [3]. Standard management is symptomatic. DMD has shown to benefit to an extent with use of steroids [4]. But, there is no known satisfactory cure and treatment involves complex, long-term health services

putting an emotional and financial burden on the families [5].

Newer therapies such as gene therapy and cell therapy are being widely explored as treatment options. Gene therapy using viral and non-viral vectors is restricted due to immune responses and safety issues [6]. Use of antisense nucleotides approach of gene therapy continues to have limitations such as poor cellular uptake, low efficacy in target tissues, risk of toxicity and high treatment cost [6]. Eteplirsen has received approval for treatment of DMD patients with a mutation of dystrophin gene amenable to exon 51 skipping. This contributes to only 13% of the DMD population and, its clinical efficacy is yet to be established [7].

Through its capacity for self-renewal and contribution to the replenishment of stem cell pool, cell therapy has the potential to slow the disease progression. Moreover, although the causative factor is genetic in origin, the progression of the disease is attributed to the loss of the regenerative capacity of muscles, making it necessary to target replenishing the resident stem cell pool to achieve therapeutic benefits. Autologous stem cell therapy is not posed with the risk of immune response. Though, the ideal cell type for transplantation in MD remains elusive, the ability of the bone marrow mononuclear cells (BMMNCs) to reach the site of muscle injury and contribute to the replenishment of stem cell pool [8] makes them anattractive alternate treatment strategy. This review aims at amalgamating existing evidence of safety and effectiveness of autologous BMMNC transplantation in MD.

Materials and Methods

We conducted an online search of published clinical studies of administration of BMMNCsas treatment in MD. Search terms included, autologous bone marrow derived cells, autologous bone marrow mononuclear cells,and autologous bone marrow derived stem cells. These search terms were combined with MD, muscle disorders, DMD, BMD, LGMD, Facioscapulohumeral Muscular Dystrophy, CMD, Dystrophinopathy, Sarcoglycanopathy. Research articles in English including all methodologies that provided the relevant data, were included. PubMed, Google and Google scholar were the databases that were searched.

Inclusion criteria

Due to lack of randomized studies evaluating the safety and/or efficacy of autologous BMMNC transplantation in MD; case reports, and case series were included. Studies that used any route of administration were included.

Exclusion criteria

Studies that involved ex vivo genetic correction of autologous BMMNCs were excluded. Also, reviews were excluded to avoid duplication. We identified one ongoing clinical study, which is being conducted to study the safety and efficacy of bone marrow derived autologous stem cells in DMD. This was excluded from the review as it is an ongoing trial and results are unknown [9].

Information gathered

Data on the year of publication, type of MD, number of patients, number of patients lost to follow up, age and gender, reported adverse events, outcome measures and the clinical results was then extracted.

Assessment of quality of the clinical studies

Quality of the studies was assessed based on factors such as presence/absence of clear and well-defined objectives, a clear description of the inclusion and exclusion criteria, justified sample size, adequate description of the methodology of intervention, outcome measures and the statistical tools used (Appendix 1, Appendix II)[10,11].

Results

Characteristics of the studies identified A total of

13 studies [12-24]were identified that met the inclusion criteria. These were published between 2012 and 2017. The intervention involved administration of autologous BMMNC by the intrathecal and intramuscular routes followed by neurorehabilitation. There were 3 open label single arm case series that included 247 patients; 1 open label study using autologous BMMNCs in neurological disorders in the pediatric population [12], another study using autologous BMMNC transplantation in patients with MD [13] and 1 open label study using autologous BMMNC transplantation in LGMDs [14].10 studies were case reports. The MDs included in the studies were; DMD, BMD, CMD and LGMD. The mean age, proportion of male-female patients and follow up duration of the open label studies could not be computed for lack of data. Mean age of the included case reports was 21.8±10.8 yearsand the mean follow-up duration was 18.5±10.3 months. All the preliminary studies met the prespecified criteria for quality and were of fair to good quality.

Side effects

Adverse events related to the procedure were observed in 3 of the studies which included nausea, spinal headache, backache, vomiting, pain at aspiration site, pain at the injection site, fatigue, pain in upper and lower limbs and neck pain [12-14]. All these were self-limiting and were relieved within a week. There were no major procedure related adverse events.

Outcome measures

Manual muscle test (MMT), Functional Independence Measure (FIM) and Brooke and Vignos scales were the most commonly used outcome measures in the studies. More objective outcome measures including, muscle strength on grip dynamometer and pinch meter were used in 3 case reports [15-17], Musculoskeletal Magnetic Resonance Imaging (MSK MRI) in 5 case reports [15-19], Magnetic Resonance Spectroscopy (MRS) in 1 case report [20] and Electromyography/ Nerve Conduction Velocity (EMG/NCV) studies in 1 case report [19]. Other outcome measures included 6-Minute Walk Test, North Star Ambulatory Assessment (NSAA), Berg Balance Scale (BBS)/ Pediatric Berg Balance Scale (PBS), Left Ventricular Ejection Fraction (LVEF) and maximum phonation duration (MPD). Characteristics of the case series and case reports are shown in Table 1.

Effect of treatment on various outcome measures

Muscle strength changes

Muscle strength as assessed on MMT, pinch strength and grip strength was used as outcome measure in 12 of the 13 studies [12-23]. Considering maintenance in muscle strength as a positive outcome, all the 12 studies reported positive outcome in muscle strength. The follow-up duration of the studies ranged from 4 months to 54 months.

Changes on Functional scales

Function as assessed on FIM was used in 10 studies [12-16,18,19,21,23,24], Brooke-Vignos scale in 5 studies [12,13,16,19,21],NSAA in 3 studies [16,21,24] andBBS/PBSin 4 studies [16,21,22,24]. One study used 6-Minute Walk Test [22] and another used MPD [16] as outcome measures. All the studies demonstrated improvement in function. Maintenance of function was considered as positive outcome to intervention. The follow-up duration ranged from 9 months to 54 months.

Radiological and electrophysiologicalchanges

5 studies used MSK MRI as an outcome measure [15-19], 1 study used MRS [20] and another used EMG/NCV studies [19] as outcome measures. We considered no further increase in fatty infiltration to be a positive treatment outcome. All the studies that used MSK MRI as outcome measure demonstrated positive outcometo intervention. Improvements were also seen on EMG/NCV [19] and MRS [20]. The follow-up duration ranged from 4 months to 24 months.

Table 1. Characteristics of studies included in the review

Author, year [Reference]	No of patients	Mean Age in years	Gender (male-M, female-F)	Type of MD	Route of administration	Follow up duration in months	Adverse events	Outcome measure	Clinical Outcome and Percentage of patients improved / maintained
Open label single-arm studies									
Sharma et al, 2012[12]	38	Not available	Not available	36 DMD; 2 CMD	Intrathecal and intramuscular	15±1	nausea, headache and backache.	MMT, FIM and Brooke & Vignos scales	Improvement in 97% patients
Sharma et al, 2013[13]	150	DMD:11.5; LGMD:34.4; BMD:27.3	DMD & BMD-130 M; LGMD-14 M &16 F	125 DMD; 20 LGMD; 5 BMD	Intrathecal and intramuscular	12±1	Headache, nausea, vomiting and backache	MMT, FIM and Brooke & Vignos scales	Improvement in 87% patients
Sharma et al, 2015[14]	59	32	39 M,20 F	LGMD	Intrathecal and intramuscular	9-54	nausea, headache, pain at aspiration site, pain at the injection site, fatigue, pain in upper and lower limbs, neck pain.	MMT, FIM	Improvement in 84-100% on MMT, 97% on FIM in 1 SCT group; 90-100% on MMT, 96% on FIM in 2 SCT group; 3 of 4 on MMT and FIM.
Case Reports: Becker's Muscular Dystrophy (BMD)									
Sharma et al, 2013[18]	1	39	M	BMD	Intrathecal and intramuscular	24	none	MMT, FIM, MSK MRI	100% improvement. Improvement on MMT, FIM; no increase in fatty infiltration on MSK MRI

Sharma et al, 2013[15]	1	28	M	BMD	Intrathecal and intramuscular	21	none	Grip strength on Dynamometer FIM, MSK MRI	100% improvement. Improvement on grip dynamometer, FIM, reduction in fatty infiltration on MSK MRI
Sharma et al, 2016 [21]	1	20	M	BMD	Intrathecal and intramuscular	9	none	MMT, NSAA FIM, BBS, Brooke & Vignos	100% improvement. Improvement on MMT, maintained NSAA, FIM, BBS, Brooke &Vignos scores
Sharma et al, 2016 [16]	1	21	M	BMD	Intrathecal and intramuscular	19	none	MPD, MMT, pinch strength, MSK	100% improvement. Increased MPD, pinch, BBS,
								MRI, FIM, BBS, NSAA, Brooke & Vignos scale, LVEF	FIMand LVEF; maintained NSAA and Brooke & Vignos scores, MSK MRI showed no increase in fatty infiltration.
Case Reports: Duchenne Muscular Dystrophy (DMD)									
Sharma et al, 2012 [17]	1	18	M	DMD	Intrathecal and intramuscular	4	none	MMT, Grip strength on dynamometer, MSK MRI	100% improvement. Improvement on MMT, grip dynamometer and MSK MRI

Sharma et al, 2014 [19]	1	9	M	DMD	Intrathecal and intramuscular	36	none	MMT, Brooke& Vignos, FIM, MSK MRI, EMG	100% improvement. Maintained strength on MMT, improvement on Brooke& Vignos, FIM scales, EMG/ NCV and no increase in fatty infiltration on MSK MRI.
Sharma et al, 2017 [24]	1	10	M	DMD	Intrathecal and intramuscular	13	none	FIM, NSAA, BBS	100% improvement. Improved FIM, and maintained NSAA and BBS scores.
Sharma et al, 2017 [22]	1	8	M	DMD	Intrathecal and intramuscular	12	none	MMT, Gower's time, pediatric BBS, 6-MWT	100% improvement. Decrease in Gower's time, maintained pediatric BBS, 6- MWD
Case Reports: Limb Girdle Muscular Dystrophy (LGMD)									
Sharma et al, 2017[23]	1	39	M	LGMD	Intrathecal and intramuscular	36	none	MMT, FIM	100% improvement. Improvement on MMT, maintained FIM
Sharma et al, 2016 [20]	1	26	F	LGMD	Intrathecal and intramuscular	9	none	MMT, MR Spectroscopy	100% improvement. Improvement on MMT, reduced area under the curve in EMCL peak and decrease in IMCL/Cr ratio.

Table 1.Stem Cell therapy: SCT; Manual Muscle Testing: MMT; Functional Independence Measure: FIM; Musculoskeletal Magnetic Resonance Imaging (MSK MRI); North Star Ambulatory Assessment: NSAA; Berg Balance Scale: BBS; Maximum phonation duration: MPD;Electromyography: EMG, Nerve Conduction Velocity: NCV; 6-MWT: 6-Minute Walk Test; Extramyocellular lipid: EMCL; Intramyocellular lipid: IMCL; Creatine: Cr

Discussion

Studies have suggested that deficiency of sarcolemmal-associated proteins in MD induce conformational changes in calcium channels resulting in increased intracellular calcium and mitochondrial dysfunction [25]. This may lead to muscle cell death and muscle weakness [1]. Recent studies have also revealed that the intrinsic defects in MD reduce the generation of the myogenic progenitors necessary for muscle regeneration [26]. The muscle wasting in MD is caused not only by the abnormal interaction between the sarcolemmal-associated proteins and calcium channels but also by the impaired regeneration resulting from the intrinsic satellite cell dysfunction. Continuous cycles of injury and repair overexposes the environment to inflammatory cytokines leading to chronic inflammation whichover time, endsin fibrosis and muscle atrophy [27,28]. Thus, effective treatment of MD will require aiming at replenishment of the stem cell pool and facilitating muscle repair. Effectiveness of cells used for transplantation depends on several factors such as obtaining sufficient number of cells, minimal invasiveness of procedure, myogenic potential of cells, ability of cells for migration and homing into the site of muscle degeneration, and ability to restore the stem cell pool [29].

Cell types for cell therapy

Several studies have used intramuscular injections for myoblast transplantation, but none of these demonstrated clinical benefits [30-42]. Muscle side populationis unable to participate in long term regeneration and currently do not contribute to being an appropriate cell source in treating MDs [8].Bone marrow derived cells are easy to isolate,are excluded from ethical concerns, areeasy to access and transplant [43], and are marked by a lower risk of immunogenecity and tumorigenicity [44,45]. Various pre-clinical studies have demonstrated that the bone marrow derived stem cells possess neurogenic potential [46] andcan differentiate into the myogenic lineage [47,48]. Also, they possess the ability to migrate to the area of muscle injury and degeneration, to repopulate the stem cell pool promoting regeneration of damaged muscle fibers [47,48] and can survive in the injected muscles for long periods of time [8].

Postulated mechanism of action of BMMNCs for treating muscular dystrophy

BMMNCs constitute a combination of cells including mesenchymal or stromal cells, hematopoietic stem cells and endothelial progenitor cells [49]. As mentioned aboveBMMNCs may exert therapeutic effects through myogenesis, neurogenesis, replenishment of stem cell pool and secretory paracrine effects [46-48,50]. The cells secrete a broad spectrum of cytokines and growth factors which promote angiogenesis, inhibition of apoptosis, anti-inflammation, immunosuppression, homing of endogenous satellite cells and regulation of specific metabolic pathways [50,51]. The muscle degeneration associated with MD is followed with an invasion of inflammatory cells such as macrophages and T-lymphocytes [52]. T-cells play a role in the fibrosis which further reduces the ability of the muscle to regenerate.Given the anti-inflammatory effect of bone marrow stromal cells, these cells may offer protection from the damage caused by T-lymphocytes [53]. Membrane-derived vesicles arising from BMMNCs may result in transcript transfer from the stem cells to the injured cells, causing the injured cells to re-enter cell cycle thus facilitating tissue repair [54,55].

Route of delivery of mononuclear cells

Intra-arterial and intravenous routes

The choice of route of administration of cells is important as it influences the outcome of treatment. Intravenous and intra-arterial route of administration results in significant filtration of cells into the lungs, kidneys and spleen and only a

small fraction of cells reaches the target tissue [56]. Intravenous administration may evoke an unwanted immune response [57].None of the clinical trials using autologous BMMNCs in MD,used this route.

Intrathecalroute

Though, MD is primarily a muscle disorder, studies have suggested possible nervous system involvement [58,59].Rosman and Kakulas [58], observed abnormalities in the brain in cases of DMD with intellectual disability.Dystrophin is a structural component of neurons in several regions in the central nervous system (CNS) [59]. Dastur and Razzak [60] studied muscle biopsy specimens of 1348 patients of which 179 patients had MD and 140 patients had anterior horn cell disorders. The study demonstrated an overlap of pathological changes in the muscles. Histological study of the muscle biopsy specimens revealed small atrophied muscle fibers suggesting a possible denervation in about one fourth of the MD patients, suggesting involvement of neural systems.Stem cells administered intrathecally are known to survive and migrate to the proximity of central nervous system abnormality.These findings support intrathecal administration of stem cells used in these studies.

In all the reviewed clinical studies, autologous BMMNCs were administered intrathecally and intramuscularly at the motor points of functionally important muscles with weakness.

Intramuscular route

The DGC is abundant at the neuromuscular junction (NMJ) and is known to be required for neuromuscular homeostasis. Defects in DGC result in impairment of neuromuscular transmission and motor end plate abnormalities [61]. Injecting the cells at motor points, which are points on the skin overlying the point at which the innervating nerve enters the muscle, ensures repair of the innervating nerve, muscle, and myoneural synapse.

Combination of rehabilitation with cell transplantation

Another feature of these studies was that the cell transplantation was followed by neurorehabilitation. A long term, low-intensity strengthening and low-load weight-bearing exercise program may cause a shift in type II fibers to type I muscle fibers which are less vulnerable to degeneration in MD [62]. In response to exercise, there is an increase in secretion of vascular endothelial growth factor (VEGF) which is known to stimulate satellite cell proliferation [63] and migration [64] thuspromoting my of iber regeneration [63,65]. Exercise also helps improve respiratory and cardiac function [66].

Clinical Evidence of safety of BMMNCs in MD

All the included studies were non-randomised, preliminary studies. All the studies, demonstrated the safety of cell therapy using autologous BMMNCs. These studies encountered no procedure related major complications. 3 studies reported minor adverse events which included headache, nausea, vomiting, backache and pain at injection site [12-14]. These were self-limiting and resolved within a week.

Clinical Evidence of therapeutic effects

Effect of intervention on muscle strength

Weakness of skeletal muscles is consistent with all the variants of MD [67]. Though at a variable rate, there is a progressive decline in muscle strength. The improvement in muscle strength on MMT [12-23] thus indicates possibledisease modifying benefits of autologous BMMNCs coupled with rehabilitation.

Effect of intervention on functional scales

Along with skeletal muscle weakness, decline in function is a common finding in all the variants of

MD [68]. There is a progressive decline in the functional capacity of patients with MD at a rate that varies among different types of MD. Improvement was seen on functional outcome measures such as FIM [12-16,18,19,21,23,24], Brooke and Vignos scales [12,13,16,19,21], BBS [16], 6-Minute Walk Test [22] and NSAA [16,21,24], thus revealing possible disease modifying benefits of autologous BMMNCs coupled with rehabilitation. BBS is sensitive to alterations in balance skills and is a reliable diagnostic and monitoring tool [69,70]. A declining score may be an indicator of increased risk of falls in adults [71]. An improvementon BBS was reported in a case of BMD [16], thus showing improved balance skills and a possible decrease in risk of falls post cell therapy. Retaining of distance on 6-Minute Walk Test was reported in a case of DMD [22] over a follow up period of 1 year. 6-Minute Walk Test decreases with age [72] in DMD, so a maintained distance on 6-Minute Walk Test may be considered of positive therapeutic value. Additionally, Sharma et al reported an improvement in LVEF from previous 36% to 45% post intervention [16] as a possible response to endurance and breathing exercises in 1 case report [16].

Effect of intervention on radiological and electrophysiological studies

MSK MRI serves as a biomarker for disease progression in dystrophinopathies and is sensitive to disease progression within a 12-month time frame [67]. Sharma et al reported decrease in fatty infiltration on MSK MRI in a case of BMD; 6 months post intervention [15] and no further increase in fatty infiltration in another case of BMD over a follow up period of 13 months [16]. In another study, comparative MSK MRI done post intervention in two of the MD patients revealed a decrease in fatty infiltration [12]. In the same study improved electrical activity was seen on EMG in 3 patients. Sharma et al reported similar improvements on MSK MRI in 6 patients and on EMG in 9 patients in a clinical trial involving 150 MD patients [13]. Theextramyocellular lipid(EMCL) and EMCL/Creatine ratio quantifies fat content in a diseased muscle [27]. A higher ratio signifies progressive fatty infiltration [27]. Decrease in the ratio with a decrease in EMCL resonance peak was seen in a case of LGMD [20], thus demonstrating a positive objective treatment outcome.

These results appear promising with all the included studies demonstrating clinical benefits spanning over a sufficient follow up duration;but none of them were controlled. Lack of control can give rise to imbalance of factors that may cause bias and uncertainty of the estimation of treatment efficacy. Most studies that evaluated treatment outcome using objective measures were case reports. Since, the primary pathophysiology in MD, is a progressive decline in muscle strength, it is one of the most important parameters to assessas outcome of a treatment. But, muscle strength was compared with natural disease history only in case reports. Case reports are valuable in generating hypothesis and a wider understanding, but they are inadequate in establishing a cause-effect relationship. Application of quantitative muscle testing and/or comparison with the natural history will be useful in providing an evidence of clinical efficacy.For a more comprehensive understanding of disease progression on different functions, comparison with natural control as well as delay in achieving certain milestone events, such as time to loss of ambulationmay be employed in future studies. Also, survival being a major concern in DMD, addressing this aspect is essential.

Conclusion

The studies included show that administration of autologous BMMNCs intramuscularly and intrathecally is safe in patients with MD. These also provide preliminary evidence of presumed efficacy of cell therapy using autologous BMMNCs coupled with standard treatment.Autologous BMMNCs possess several

benefits including ease of access, availability, ability to repopulate the stem cell pool and, myogenic and neurogenic potential, addressing the underlying pathology of the disease and may serve as a useful interim treatment option in MD. More rigorous research methodologies are needed to establish its efficacy in MD.

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Appendix I

Quality Assessment Tool for Case Series Studies

Criteria	Reference [9]	Reference[10]	Reference[11]
1. Was the study question or objective clearly stated?	✓	✓	✓
2. Were eligibility/selection criteria for the study population prespecified and clearly described?	✓	✓	✓
3. Were the participants in the study representative of those who would be eligible for the test / service / intervention in the general or clinical population of interest?	✓	✓	✓
4. Were all eligible participants that met the prespecified entry criteria enrolled?	✓	✓	✓
5. Was the sample size sufficiently large to provide confidence in the findings?	×	×	×
6. Was the test / service / intervention clearly described anddelivered consistently across the study population?	✓	✓	✓
7. Were the outcome measures prespecified, clearly defined, valid, reliable, and assessed consistently across all study participants?	✓	✓	✓
8. Were the people assessing the outcomes blinded to the participants' exposures/interventions?	×	×	×
9. Was the loss to follow-up after baseline 20% or less? Were those lost to follow-up accounted for in the analysis?	✓	✓	✓
10. Did the statistical methods examine changes in outcome measures from before to after the intervention? Were statistical tests done that provided p values for the pre-to-post changes?	×	✓	✓
11. Were outcome measures of interest taken multiple times before the intervention and multiple times after the intervention (i.e., did they use an interrupted time-series design)?	×	×	×

Quality Rating (Good, Fair, or Poor): Less than 50%-Poor, 51-70%-Fair, and 71-90%-Good

Appendix II

Checklist for assessing the quality of a case study report [79]

1. Is this report easy to read?
2. Does it fit together, each sentence contributing to the whole?
3. Does this report have a conceptual structure (i.e., themes or issues)?
4. Are its issues developed in a series and scholarly way?
5. Have quotations been used effectively?
6. Has the writer made sound assertions, neither over- or under-interpreting?
7. Are headings, figures, artefacts, appendices, indexes effectively used?
8. Was it edited well, then again with a last minute polish?
9. Were sufficient raw data presented?
10. Is the nature of the intended audience apparent?
11. Does it appear that individuals were put at risk?
12. Is the case adequately defined?
13. Is there a sense of story to the presentation?
14. Is the reader provided some vicarious experience?
15. Has adequate attention been paid to various contexts?
16. Were data sources well-chosen and in sufficient number?
17. Do observations and interpretations appear to have been triangulated?
18. Is the role and point of view of the researcher nicely apparent?
19. Is empathy shown for all sides?
20. Are personal intentions examined?
21. Is the case study particular?
22. Is the case study descriptive?
23. Is the case study heuristic?
24. Was study design appropriate to methodology?